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C. THURSTAN HOLLAND in *The British Journal of Radiology*

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AN EARLY HUMAN OVUM (THOMSON) *IN SITU*

By P. N. B. ODGERS

From the Department of Human Anatomy, University of Oxford

In March 1913 the late Prof. Arthur Thomson received the uterus, which contained this ovum, with its adnexa from Prof. H. M. Turnbull of the London Hospital. He briefly demonstrated it to the Anatomical Section of the XVIIth International Congress of Medicine, which was held in London in the summer of that same year, but he never published any detailed description of it. Some five years ago he suggested to me that it was well worth further examination, but it was not until after his death that the slides of this specimen with his serial microphotographs and notes about it were brought to my notice. I shall call it in this paper after his name, and I hope that, if it is ever referred to in the future, it may be known as the Thomson ovum.

Prof. Turnbull discovered it in the decidua of the posterior wall of the uterus of an unmarried woman, aged 20, who died 5 hours after severe burns on 4 March 1913. The right ovary contained a corpus luteum, which measured $24{\cdot}5 \times 17{\cdot}5$ mm. The woman's menstrual history was as follows. It first occurred at 15 years of age and had always been regular. Her last period commenced on 3 February, exactly 29 days before her death. No dates of coitus were available. The post-mortem examination took place 26 hours after death. The specimen was fixed in 10 per cent. formalin and cut into sections 10 μ thick.

From the menstrual history the maximal age of this ovum must be about 16 days. The inside measurements of the blastocyst are $2{\cdot}1 \times 1{\cdot}51 \times 0{\cdot}7$ mm.; the embryonic disc measures $0{\cdot}26 \times 0{\cdot}31$ (?) $\times 0{\cdot}16$ mm. (?).

In the following list it is placed among its contemporaries. Dr S. Zuckerman has kindly computed for me from the inside measurements of the blastocyst cavity a rough estimate of its cubic capacity in the several ova, the cavities being regarded for this purpose as perfect spheres, of which the diameters were taken as the mean of the dimensions given. These figures are added in a separate column.

	Inside measurements in mm. of blastocyst cavity	Capacity in c.mm. of blastocyst cavity
(1) Linzenmeier (1914)	$0{\cdot}61 \times 0{\cdot}52 \times 0{\cdot}75$	0·13
(2) Stieve (1931)	$0{\cdot}44 \times 1{\cdot}08 \times 0{\cdot}42$	0·14
(3) Peters (1899)	$1{\cdot}6 \times 0{\cdot}9 \times 0{\cdot}8$	0·70
(4) v. Möllendorff Ei OP (1921)	$1{\cdot}5 \times 1{\cdot}15 \times 1$	0·94
(5) Fetzer (1910)	$1{\cdot}6 \times 0{\cdot}9$	1·02
(6) Falkiner (1932)	No measurements: estimated age 15 days	
(7) Tennant & Ramsey (1934)	$2{\cdot}17 \times 1{\cdot}4 \times 0{\cdot}6$	1·41
(8) Thomson (1913)	$2{\cdot}1 \times 1{\cdot}51 \times 0{\cdot}7$	1·55
(9) Herzog (1909)	$2{\cdot}32 \times 1{\cdot}2 \times 0{\cdot}8$	1·56
(10) Heine & Hofbauer (1911)	$2{\cdot}38 \times 0{\cdot}98 \times 1{\cdot}2$	1·84
(11) Schlagenhaufer & Verocay (1916)	$2 \times 1{\cdot}6 \times 1$	1·89
(12) Jung (1907)	$2{\cdot}5 \times 2{\cdot}2 \times 1{\cdot}1$	3·78

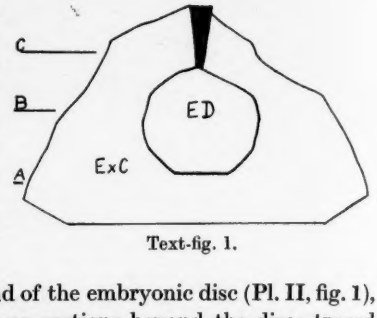
As is seen in the general view (Pl. I, fig. 1), the blastocyst cavity and the trophoblast are well preserved, but the embryonic disc, particularly the ectoderm, has suffered a good deal of disorganization, the amniotic cavity being partly obscured by cellular detritus. While this forbids any detailed description of the disc itself, I wish to draw attention here to:

(1) the formation of the extra-embryonic coelom, and

(2) the wall of the yolk sac.

I. THE EXTRA-EMBRYONIC COELOM

On either side of the amnio-embryonic vesicle a space is seen (Pl. I, fig. 2), the one on the left being triangular in section, while the right one appears more quadrangular in shape. These are marked off from the rest of the blastocyst cavity by a regular layer of mesothelial cells, which vary in different sections in their number and their shape, most of them being round, while a minority are flatter and spindle-shaped. They are partially filled by coagulum similar to that of the magma. If the almost symmetrical channels thus formed are traced in one direction, they are seen to coalesce and surround this end of the embryonic disc (Pl. II, fig. 1), the common channel extending for thirteen sections beyond the disc: traced in the opposite direction they are observed to narrow and finally disappear, the right before the left, sixteen sections from the disc, but here they are always separated from each other by a strand of mesenchyme and never join (Pl. II, fig. 4).



Text-fig. 1.

Text-fig. 1 is a diagram drawn roughly to scale to show the disposition of these channels (*ExC*) around the embryonic disc (*ED*) viewed from the dorsal aspect. The letters *A*, *B*, *C* correspond to the levels of the microphotographs (Pl. II, fig. 1; Pl. I, fig. 2; and Pl. II, fig. 4). While, as I have said, these channels are well marked out from the rest of the blastocyst cavity for the most part, there are in the case of either of them gaps in their parietes where they become continuous with the adjacent magma spaces. When traced in serial sections, such spaces are seen to be increasingly outlined by mesothelial cells so that eventually they appear as little bays in the lateral wall of the channel which is thereby correspondingly enlarged.

The only other specimen which shows anything exactly like this one is the Peters ovum, but that of Tennant & Ramsey presents a condition very comparable to it. The former of these was re-examined by Prof. O. Grosser in 1908, and he has been good enough to send me his microphotograph of this ovum and very kindly permits me to reproduce it here for comparison (Pl. II, fig. 2).

The only differences in the appearance presented by these two sections are

that in the Peters ovum (1) the lateral wall of the right channel has been torn away, (2) the containing wall of the intact left channel appears to be more cellular, and (3) it extends on to the ventral wall of the yolk sac, while in the Thomson ovum, although they are wider, these channels are confined to the neighbourhood of the amnio-embryonic vesicle. Grosser described the channel in the Peters ovum as horseshoe-shaped; it surrounds the cranial end of the disc as a small fissure. This is rather different from the wide communication presented by the present specimen.

Similarly, Tennant & Ramsey described "a space around the embryonic mass which is limited by a single layer of flattened mesodermal cells having the appearance of an endothelium (Pl. II, fig. 3). This layer not only surrounds the space but likewise completely encloses those surfaces of the embryonic mass not directly attached to the mesenchyme." In their specimen, too, the space is horseshoe-shaped, as must be inferred from the fact that they found that "the left end of the embryonic disc stops bluntly and is entirely free of mesothelial attachment while the right is completely encased in mesenchyme".

The three ova, Thomson, Peters and Tennant & Ramsey, seem to represent three stages in the development of these channels. In the first they border the amnio-embryonic vesicle only, in the second they have extended on to the lateral side of the yolk sac, while in the last they practically enclose the yolk sac on all sides. Here, although the authors describe *a* space, I think there is a fine strand in the microphotograph they reproduce between the mesothelial layer covering the ventral wall of the yolk sac and the parietes of the space to the right of the midline which may indicate its bilateral origin (Pl. II, fig. 3), while in their reconstruction they show the ventral surface of the embryo as almost entirely free of mesenchyme, "with the exception of a small band of attachment extending about half way along this surface from the encapsulated end".

In 1908 Keibel & Elze and Grosser described these channels as the commencement of the extra-embryonic coelom. Grosser (1924) suggested that they gradually grow ventrally round the yolk sac, their opposed walls possibly forming, when they meet, the "distaler Nabelblasenfaden", the mesodermal strand which may connect the ventral pole of the yolk sac with the chorion. Further, he thought that these exocoelomic channels extend lateralwards by taking up the magma spaces nearest to them so that eventually the whole cavity of the blastocyst becomes the extra-embryonic coelom. He wondered if the loss of the lateral wall in the left channel in the Peters embryo might not be due to its having already broken down so that on this side the exocoelom was continuous with the intercellular spaces of the magma. I have mentioned above that in the Thomson ovum there is evidence of the commencement of the same process, while Tennant & Ramsey wrote that in the specimen they described "on the surface towards the main chorionic cavity the continuity of this membrane-like structure is less well maintained and irregularly distributed openings in it offer probable avenues of communication between the larger and the smaller spaces".

These three ova are peculiar then in being the only ones so far described which exhibit definite symmetrical coelomic channels.[1] The Fetzer (Grosser, 1924) and Herzog (Lewis, 1912) ova and the much more advanced embryo described by Waterston (Mall, 1916) have all been considered by these different observers to show something similar, but I would suggest that at all events the illustrations, which accompany the descriptions of these specimens, are far from convincing from this point of view. In all other specimens the whole of the blastocyst cavity seems to represent the extra-embryonic coelom (Bryce, 1924–5). An accumulation of magma fluid appears according to this author's account near its centre close to the embryonic disc, which gradually leads to the stretching and absorption of the mesodermal bands traversing it and to the isolation of the embryo, so that ultimately all the magma spaces run together and the whole of the magma cavity becomes the exocoelom. Grosser (1913) and v. Möllendorff (1921) both suggested that there are thus two alternative ways in which this coelom may be formed.

It is, however, perhaps pertinent to observe that in the Peters ovum the trophoblast is already lined with "morula" mesoderm (Stieve, 1926), which also covers the ventral wall of the yolk sac beyond the limits of the exocoelomic channel. The Thomson ovum presents the same appearance, although the mesodermal cells covering the yolk sac are few. In either of these cases, apparently, a gradual liquefaction of the magma would result in a perfect coelom lined with mesoderm without the help of the mesothelium of the channels at all.

With regard to the later fate of these exocoelomic channels there is only Grosser's surmise to guide us, and one cannot help thinking that their formation as seen in the three specimens in which they occur serves no useful purpose. In any case their appearance in the Tennant-Ramsey and Thomson ova prove that the Peters ovum is no longer quite exceptional.

II. THE YOLK SAC

As seen in Pl. I, fig. 2, this appears as a space beneath the amnio-embryonic disc, outlined from the rest of the magma cavity by a thin membranous wall. It is flattened dorsoventrally. On the left side, its ventral wall is connected by an irregular strand of mesenchyme cells to the chorion, but this is the only evidence of a commencing "Nabelblasenfaden" so obvious in the Schlagenhaufer-Verocay specimen. While the dorsal wall of the yolk sac beneath the embryonic disc has a lining of cubical entodermal epithelium its lateral and ventral walls are formed by a sparse layer of flattened spindle-shaped cells mesothelial in character. These latter appear to be similar to many of the cells lining the extra-embryonic coelomic channels, and the coagulum within the yolk sac seems to be exactly like that filling these

[1] Since this was written K. Hiramatsu (*Fol. Anat.* Japon, 1936, Bd. XIV, p. 15) has published an account of the ovum, Ei-Ando. In this he describes two small cavities on either side of the dorsal portion of the yolk sac, which he thinks correspond to these exocoelomic channels.

channels and the magma spaces. Clothing the lateral walls and extending on to the ventral angles of the sac are a few round mesodermal cells. It is interesting to compare the condition found here with that noted or illustrated in the other ova in the list given above.

Linzenmeier. There is no mention of this point in the text, but the accompanying illustration shows flattened cells on the ventral side of the yolk sac with a cubical entoderm under the embryonic disc.

Stieve. "The yolk sac is outlined by spindle-shaped mesoderm cells, while the entoderm is only represented by a single layer beneath the ectoderm of the disc."

Peters. Here the whole sac is lined by a cubical epithelium of the same type all round (Pl. II, fig. 2). In Peters' original drawing the cells on the ventral wall are more irregularly spaced than are those beneath the disc.

v. Möllendorff, Ei OP. "In its whole circumference the yolk sac is lined with markedly flattened cells with lens-shaped nuclei." His figure shows a condition very much like that I have described in the Thomson ovum.

Fetzer. The sac is lined except beneath the disc with a "thin low entoderm". Sporadically the cells are larger, cubical or short cylindrical in type (Fetzer & Florian).

Falkiner. He gives no description of this point, but his illustration shows the lining of the yolk sac very much like that in the Thomson ovum.

Tennant & Ramsey. "The yolk sac is lined with entodermal cells, which are cuboidal on the dorsal surface and flattened on the ventral surface" (Pl. II, fig. 3).

Herzog. Lewis (1912) described the sac in this ovum thus: "Over the greater part of it the entoderm forms a very thin layer resembling endothelium."

Heine & Hofbauer. The yolk sac is lined by a single layer of flattened cells except under the disc.

Schlagenhaufer & Verocay. The yolk sac is lined throughout with entoderm.

Jung. The yolk sac shows a simple layer of flat entoderm cells.

With the Peters and Schagenhaufer ova standing out as exceptions this analysis confirms Bryce's (1924–5) statement that in the first instance the yolk sac is always lined with flattened endothelial-like cells. In older embryos, as Grosser (1924) pointed out, there is a complete change; in these (e.g. Rossenbeck, Grosser and Graf Spee ova) while the peripheral part of the sac is lined with cuboidal cells the portion of it beneath the disc has the flatter type of epithelium. Similarly Florian & Hill (1934–5) described the yolk sac wall in their specimen—"while the endoderm of its cranial and lateral walls is formed of flattened cells, that of its ventral wall is distinctly higher."

In attempting to explain these differences Stieve interpreted the appearance presented by the ovum (Werner) he described as follows. The future yolk sac is here represented by an enlarged magma space lying beneath the embryonic disc and outlined by mesoderm cells. Later the entoderm of the disc must gradually grow ventrally and surround this space on its inner aspect and so form a definite yolk sac. He followed Corning (1925), who described the entoderm in the human ovum as beginning as an entodermal plate—the lowest

layer of cells of the embryonic disc—rather than as an entodermal vesicle. In favour of such a conception are, I think, the following points:

(1) It brings the formation of the human yolk sac in line with that which has been found to occur in most other mammals (Stieve). The only observations in comparative embryology, as far as I am aware, which suggest the development of the yolk sac by the dehiscence or vacuolization of a primitively solid entodermal mass are those of Hubrecht (1889–90) in *Erinaceus* and Hill (1932) in *Nasalis larvatus*.

(2) The entodermal vesicle of the Teacher-Bryce ovum (TB. I.) lying in the magma and divorced from the amnio-embryonic vesicle might very well be described as a magma space (Stieve). Bryce (1924–5) himself described it as being lined with flattened cells precisely like those forming the wall of the sac in Linzenmeier's embryo.

(3) In the Tennant-Ramsey ovum the cubical entoderm appears to be surrounding the yolk sac and to have left only a portion of its ventral wall lined by flattened cells (Pl. II, fig. 3).

(4) In nearly all the early ova the yolk sac, lined with flat mesothelial-like cells, is filled with a loose coagulum which seems to be indistinguishable from that of the magma spaces (Triepel, 1916). On the other hand Schlagenhaufer & Verocay found in their ovum that the contents of the yolk sac stained more deeply with eosin than did that of the magma. Tennant & Ramsey described the yolk sac in their specimen as "partially filled by pink-staining finely granular material, while in other sections large yolk granules are present in the cavity". Peters figured some drops of (?) yolk-like material in his original drawing of the yolk sac in his ovum. In all these three instances, as has been noted already, the sac is lined more or less completely with a cubical entoderm. One might, therefore, suggest that there is some correlation between the character of the contents of the yolk sac and of its cellular lining.

The appearance of the yolk sac in the Thomson ovum is very much like that shown in Stieve's illustration. On Prof. J. P. Hill's advice I ventured to send the serial microphotographs of this ovum to Prof. Grosser, and he was kind enough to give me his opinion on this point after examining them. His criticism of both the Thomson and the Stieve specimen is the same. In both, according to him, the entodermal lining of the yolk sac has disappeared except under the disc. I confess I think this explanation is a difficult one. Allowing that 26 hours elapsed between the woman's death and the fixation of the Thomson ovum, the entoderm could scarcely have vanished in this time without leaving a trace. If the cells had been shed, they or their debris should surely still be found in the yolk sac, unless its walls were broken. But the yolk sac appears to be intact, filled with coagulum, but containing only an occasional detached cell. If there had been a dehiscence in its wall, the fluid content would certainly have escaped before its cellular lining. Further, as I have shown above, in most of the early ova the cells lining the yolk sac are described as being of this same character, and, while in different specimens they vary

somewhat both quantitatively and qualitatively, most embryologists would follow Bryce in describing them as flattened and endothelial-like. But, if one accepts Grosser's explanation for the Stieve and Thomson ova, is one to condemn others of about the same age which show a similar condition? One is left apparently with two alternatives. There is, on the one hand, the view generally held that these mesothelial-like cells lining the early yolk sac, so similar to many of those outlining the exocoelomic channels and to those scattered elsewhere throughout the blastocyst cavity, grow eventually into the cubical or low columnar entoderm of the older sacs. On the other hand, there is the conception of Corning and Stieve of a yolk sac originally preformed as a magma space and outlined in mesoderm, which later acquires an epithelial lining from the growing margins of an entodermal plate. This latter idea apparently has much to recommend it and I should prefer myself to interpret this specimen as rather corroborating it.

While I realize to the full the weight that rightly should be attached to Prof. Grosser's opinion, I would suggest that the appearance of the cellular lining of the yolk sac in this ovum is not so remarkable.

SUMMARY

1. The interior measurements of the blastocyst cavity in the Thomson ovum are $2{\cdot}1 \times 1{\cdot}51 \times 0{\cdot}7$ mm.; its maximal age is about 16 days.

2. The extra-embryonic coelom is here seen to be commencing as fairly symmetrical channels on either side of the amnio-embryonic vesicle. A comparable appearance to this has been observed previously only in the Peters and Tennant-Ramsey ova.

3. The yolk sac has a lining of cubical entoderm beneath the disc; elsewhere it is merely outlined by a sparse layer of spindle-shaped mesothelial-like cells. This condition is shown not to be exceptional in the earlier ova and is held to corroborate Stieve's conception of an entodermal plate spreading ventrally to form the proper entodermal wall of the yolk sac as seen in later embryos.

I am very much indebted to Prof. J. P. Hill for his kindness in examining the slides of this specimen, and to Prof. O. Grosser for giving me his opinion on the serial microphotographs of it, and for his kind permission to reproduce his own photograph of the Peters ovum. I must also thank the publishers of *Surgery, Gynecology and Obstetrics* for allowing me to reproduce the illustration, Pl. II, fig. 3, which appeared originally in that journal. The other microphotographs are the work of Mr W. Chesterman of this Department.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. The section photographed is through the middle of the blastocyst cavity (× 44).

Fig. 2. The same section as fig. 1 (× 100). This shows the exocoelomic channels, *ExC*, on either side of the embryonic disc and ventral to this the yolk sac, *YS*. Above the disc is the amniotic cavity obscured by cellular debris which partially fills it. This section corresponds to the plane lettered *B* in Text-fig. 1.

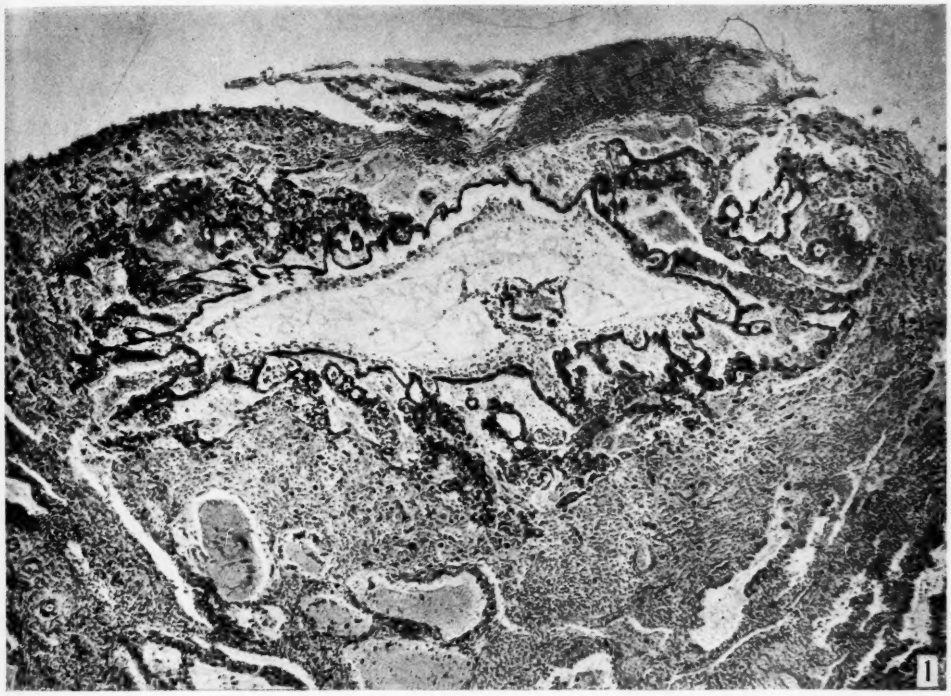
PLATE II

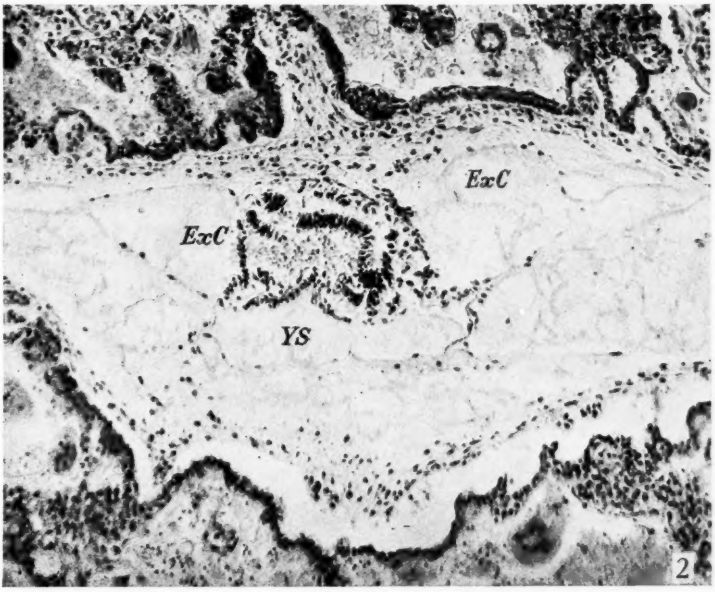
Fig. 1. A section (× 100) 20 μ beyond one extremity of the embryonic disc. It corresponds to the plane *A* in Text-fig. 1 and shows that the two exocoelomic channels have joined round this end of the disc.

Fig. 4. A section (× 100) 20 μ beyond the opposite extremity of the disc showing the two channels still separate from each other. This corresponds to the plane *C* in Text-fig. 1.

Fig. 2. A photograph of a section (× 100) of the Peters ovum, kindly sent me by Prof. O. Grosser. It shows the exocoelomic channel on the left intact, bordering the embryonic disc and the yolk sac, while that on the right has only its medial wall left.

Fig. 3. A section (× 150 ?) of the Tennant-Ramsey ovum (reproduced by courtesy of the Editors of *Surgery, Gynecology and Obstetrics*). It shows the amniotic cavity, the embryonic disc and the yolk sac surrounded on either side and ventrally by the extra-embryonic coelom.





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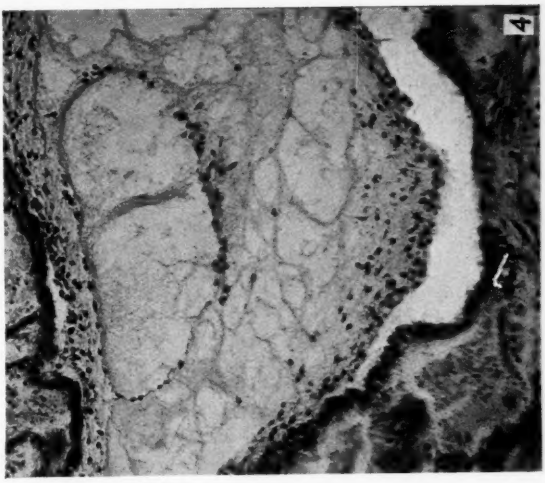

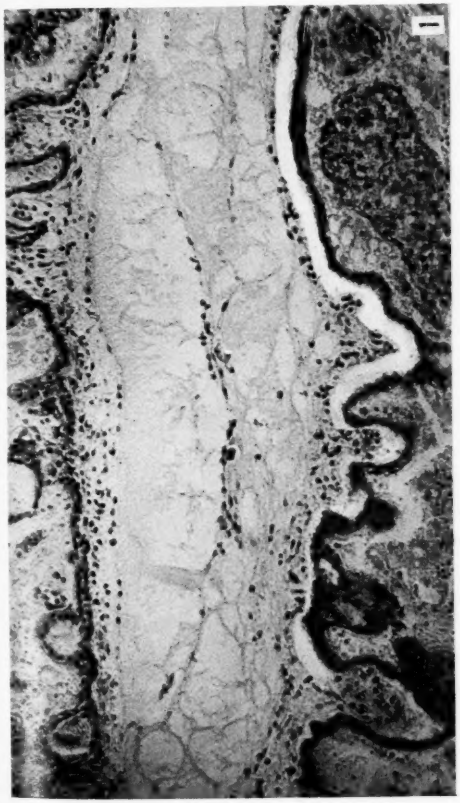

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A HUMAN EMBRYO OF TWENTY-FIVE SOMITES

By CECIL M. WEST
University College, Cardiff

The specimen about to be described was kindly given to me by Dr Jethro Gough, of the Pathological Department, Welsh National School of Medicine. It was obtained at a post-mortem examination of a woman, aged 39, who died of a strangulated hernia. The post-mortem examination was made 47 hours after death; there is, however, little probability that the material had suffered much from this lapse of time, for the body was kept in an efficient refrigerator, which fact, combined with the season of the year, January, lessens the likelihood of decomposition having set in, and Dr Gough tells me that he noticed no evidence of this when making the autopsy.

TECHNIQUE EMPLOYED ON MATERIAL RECEIVED

The material received from Dr Gough consisted of the uterus, ovaries and tubes. The uterus had been opened in front along the middle line, and there was thus exposed on the anterior wall and near the left cornu a chorionic vesicle, which was unopened.

There was a corpus luteum in the left ovary, which had been opened, and a hydatid cyst was present near the ovarian fimbria of the left side. The whole specimen was placed in 4 per cent formalin for a few days, and then the chorionic vesicle was removed from the uterus and opened, and an apparently normal embryo was exposed.

The embryo, enclosed in the amnion, was lying on its left side; the yolk stalk passed out towards the right and the body stalk towards the left; the tail of the embryo was twisted towards the right.

The amnion was then removed and the embryo, with the body stalk and the adjacent part of the chorion, was embedded in paraffin and cut into sections of 10 μ in thickness, which were stained on the slides with Ehrlich's haematoxylin and orange G.

Every section was then photographed on to bromide paper at a magnification of $\times 100$, and many reconstructions, both graphic and of wax, were made of the embryo as a whole and of its various regions.

In order to obtain the correct orientation for the reconstructions the following method was adopted: the sections had been fixed to the slides with a solution of chromatized gelatin, and after the removal of the paraffin with xylol, the sections were stained and it was found that the edges of the paraffin block could be easily identified as they had slightly taken up the haematoxylin stain; provided the paraffin block has been trimmed square, a satisfactory

method of orientation is thus easily accessible; in addition to this method use was made of photographs which had been taken of the embryo before, and after, its removal from the chorionic sac.

AGE OF THE SPECIMEN

There was no menstrual history, so that in estimating the age comparison must be made with embryos for which more definite data are available. Recent work, in Baltimore, by Streeter (1932, 1933), Hartman and their colleagues, has made it possible to determine the true ovulation age and rate of development in embryos of the monkey, *Macacus rhesus*; and while the two genera—man and monkey—differ in form and in rate of differentiation and growth, it has nevertheless been found that such differences do not become appreciable until the end of the second month; so that, for the first 6 weeks, the known ages of *Macacus rhesus* may be transferred to human embryos of corresponding developmental stages. Streeter has thus estimated the ovulation ages of certain well-known human embryos, and has found that the first somites make their appearance on the 22nd day and that differentiation of the somites occurs rapidly, 1, 5 and 8 somites being found on the same day; a 6·5 mm. embryo, with triangular fin-like arm buds and beginning leg buds was obtained on the 27th day.

The present specimen has 25 somites and very early limb buds and may thus be between the 22nd and 27th days; taking into account the rapidity of development at this stage, an ovulation age of 24 days would, I believe, be a fair estimate for this specimen.

From published data on menstrual history it is possible to work out the ovulation age of certain other embryos which resemble, in general, the present specimen; for example, embryos described by Atwell, 17 somites (1930), Watt, 17–19 somites (1915), Davis, 20 somites (1923), Girgis, 22 somites (1926), Johnson, 24 somites (1917), Waterston, 27 somites (1914). Basing the calculation of the ovulation age on the assumption that ovulation has occurred on the 14th day after the commencement of the last menstrual period, I find that this series of embryos covers a range of ages from 19 to 32 days, and of developmental stages from 17 to 27 somites; such ages do not fit in exactly with Streeter's findings, but they are based on menstrual histories and on the assumption that menstruation has been regular and that ovulation has occurred on the 14th day after the commencement of the last period. Whilst some observers would fix a more or less hard and fast date for ovulation, others would consider the date to be more variable; for example, Hain (1934) has shown in an individual in whom intermenstrual bleeding was recorded for a period of over 2 years that, if such intermenstrual bleeding may be considered indicative of ovulation, then ovulation may occur as late as, and after, the 20th day from the beginning of the previous period, and that there may be more than one ovulation in a cycle; there may thus be great variation in ovula-

tion ages as estimated from menstrual histories, and this emphasizes the importance of Streeter's and Hartman's work.

THE UTERUS AND EMBRYONIC MEMBRANES

The uterus, after fixation, measured 96 mm. from fundus to external os, and 67 mm. from side to side at the widest part of the body; the walls were 12 mm. thick.

The chorionic vesicle was removed intact from the uterus and was covered completely with villi; it was rather flattened and measured 18×15 mm.

The part of the chorion, at the attachment of the body stalk, that was cut out and sectioned with the embryo was carefully examined; the general appearance is very like that shown in Grosser's Fig. 107, p. 133, in Keibel & Mall (1910), of a section through the chorion of an aborted ovum of 1 month. Each villus and its branches are usually covered with two layers of cells: an outer layer in which cell boundaries are not well defined, and in which the nuclei are flattened, loosely spaced and often faintly stained; and an inner layer in which cell boundaries are defined, and in which the nuclei are round, closely packed and deeply stained; these two layers of the chorionic epithelium are the syncytiotrophoblast and cytotrophoblast respectively. Here and there it is quite impossible to make out any cellular structure whatever in the syncytiotrophoblast, whilst in some places the nuclei of this layer can be faintly discerned.

When the final ramifications of a villus are reached it is found that the syncytiotrophoblast fades away or disintegrates, and the cytotrophoblast becomes spread out in a sheet so that the cells of which it is composed are seen in surface view instead of in section; the cells, too, appear to increase in size and their boundaries become very sharp, and the sheet of cells forms a beautiful mosaic. When the cytotrophoblast of two adjacent villi thus becomes spread out into sheets the syncytiotrophoblast dips in between the sheets and gives the appearance of being an intrusion (Pl. I, fig. 4); similarly a part of the intervillous space comes to be included in the sheet and contains maternal blood and sometimes some fibrinous material. These intrusions into the sheet are composed of masses of protoplasm without any cell boundaries and with small, numerous and deeply stained nuclei which stand out very prominently from the lightly stained and well-defined cells of the trophoblast sheet; these sheets are the "cell islands" of Grosser. But there are to be found in the sheets and in the intervillous spaces other protoplasmic masses of an appearance quite different from that of the intrusions mentioned above; these are small masses of protoplasm, of any shape, homogeneous in appearance, rather opalescent and stained slightly pink, with crowds of brightly stained nuclei arranged usually in a circlet or in a rosette (Pl. I, fig. 4); these are the "giant cells" of Grosser, and they are either protrusions or actually detached portions of syncytiotrophoblast, and they seem to be very similar to the "proliferation nodes" to which also Grosser refers.

The mesodermal core of the villi has undergone some shrinkage from the overlying trophoblast; it contains the subdivisions of the umbilical vessels, and is composed of a loose tissue, the cells of which are of an irregular polygonal or stellate shape with round, oval or spindle-shaped nuclei; there are a few of the large cells of Hofbauer, but they are not at all numerous.

Teacher (1924), in his account of the embryo T.B. 2, describes a condition which is just like what I find in this specimen; he writes, p. 172, that the syncytium "ends a little beyond the tip of the villus leaving the cytotrophoblast uncovered, and the latter fuses with that of neighbouring villi forming a complex structure tunnelled here and there by passages for the maternal blood". Coventry (1923), in an account of the placenta of the Guinea baboon at the 4th month, writes of trophodermic cell islands being completely covered with a layer of syncytium continuous with that of the villus to which they are attached; comparing this with the condition in the human subject, he states that the trophoblastic masses in man are not covered with a continuous layer of syncytium, but that it is usual to find syncytial masses and buds scattered over the surface of the masses, and he suggests that the syncytium is at first stretched out over the developing cell mass and then, its elasticity failing, it becomes broken up into patches; this is just the impression that I get from a study of this specimen, and it seems to correspond with the condition described by Johnson (1917) in an embryo of 24 somites. The line of attachment of the amnion to the body wall follows closely the ventral borders of the two umbilical veins, and where these enter the heart the lines of reflection join just caudal to the heart and cranial to the yolk stalk, with the result that the heart is included within the amniotic cavity and the yolk stalk is not; the condition is very similar to that shown by Politzer & Sternberg (1930) in an embryo of 2·7 mm. in length and with 25 somites. The amnion covers the caudal aspect of the body stalk and is just beginning to encroach on the ventral aspect; it is most closely applied to the body of the embryo in the forebrain and tail regions, whereas it stands out at some distance from the rest of the brain and from the dorsal surface of the body, though it does follow to a slight extent the dorsal concavity of the body.

Filmy strands passed from the outer surface of the amnion and of the yolk sac to the inner surface of the chorion, but they could be removed from both structures without causing any damage. I am unable to describe the structure of these strands, but they seem to be a fairly common feature in young chorionic vesicles and are, presumably, remains of the extra-embryonic mesoderm which have persisted after the formation of the extra-embryonic coelom.

The amnion is a very thin bilaminar structure and, since most of it was removed before the embryo was sectioned, it can be studied microscopically only at the cut edges of the lines of reflection; at such places the two layers of which it is composed can be seen well; near the line of reflection between heart and yolk stalk the amnion is a little thicker than elsewhere, as was described

by Johnson (1917), and here it is possible to recognize a small amount of mesenchyme between the true mesodermal and ectodermal layers.

THE EMBRYO

External form. The embryo was cut into 297 sections, each 10 μ thick, and since the sections are almost horizontal, the embryo measures nearly 3 mm. in length.

The general form of the embryo can be estimated best from the photographs (Pl. I, figs. 1 and 2) and a lengthy description will therefore not be given, but the following points may be noticed: the heart is prominent and causes a swelling larger on the right than on the left, as has been noted by other observers in specimens of about this stage; the roof of the hindbrain is already thin and was sufficiently transparent to allow the neuromeres to be seen through it; there is a concavity of the dorsal body wall just caudal to the attachment of the yolk stalk, and herein lies the main difference between this specimen and van den Broek's (1911) embryo A, and an embryo with 25 somites referred to by Politzer & Sternberg (1930), both of which are bent in a gentle and continuous curve; the ventral body wall is widely deficient from the caudal margin of the heart to the cranial border of the body stalk, and the gut, suspended by its mesentery and with the yolk stalk opening from it, is thus clearly visible between the rounded elevations of the umbilical veins, just as described by Politzer & Sternberg; a very slight rounded elevation marks the position of the forelimb bud, and there is just a suspicion of a hindlimb bud. Various theories have been given to account for the dorsal concavity of the body; Bartelmez & Evans (1926) suggest collapse of the yolk sac during dehydration as a possible cause; Atwell (1930) suggests factors in operation during life, such as turgidity of the yolk sac, rapid increase in growth of the two ends of the body and the difficulty in accommodating both heart and yolk sac on the ventral side of the body without there being some bending. There is no doubt that these curves do appear opposite the attachment of the yolk sac, and they do tend to be more marked in the earlier stages when the yolk sac is relatively large and the body wall frail and slender; a dorsal concavity is present, for example, in nearly all the embryos, of from 1 to 16 somites, described by Bartelmez & Evans (1926), in Wilson's (1914) embryo H 98, of 1·27 mm., in Ingalls' (1920) embryo of 1·38 mm., in Dandy's (1910) embryo of 2 mm.; there is a slight concavity in Atwell's (1930) embryo of 2·81 mm., in Watt's (1915) embryo of 3·35 mm.; it is very slight in an embryo of 5 mm. described by Politzer & Sternberg (1930), while it is absent in embryos of 2·7, 4, 6, 7 mm. described by the same authors, and in Thompson's (1907) embryo of 2·5 mm. I believe that in the present specimen the concavity was developed as a result of factors in operation during life, for it could be seen as soon as the chorionic sac was opened and when the embryo had been subjected to no interference other than fixation in 4 per cent formalin.

I believe that such dorsal curvatures must be regarded as a normal variation in early developmental stages.

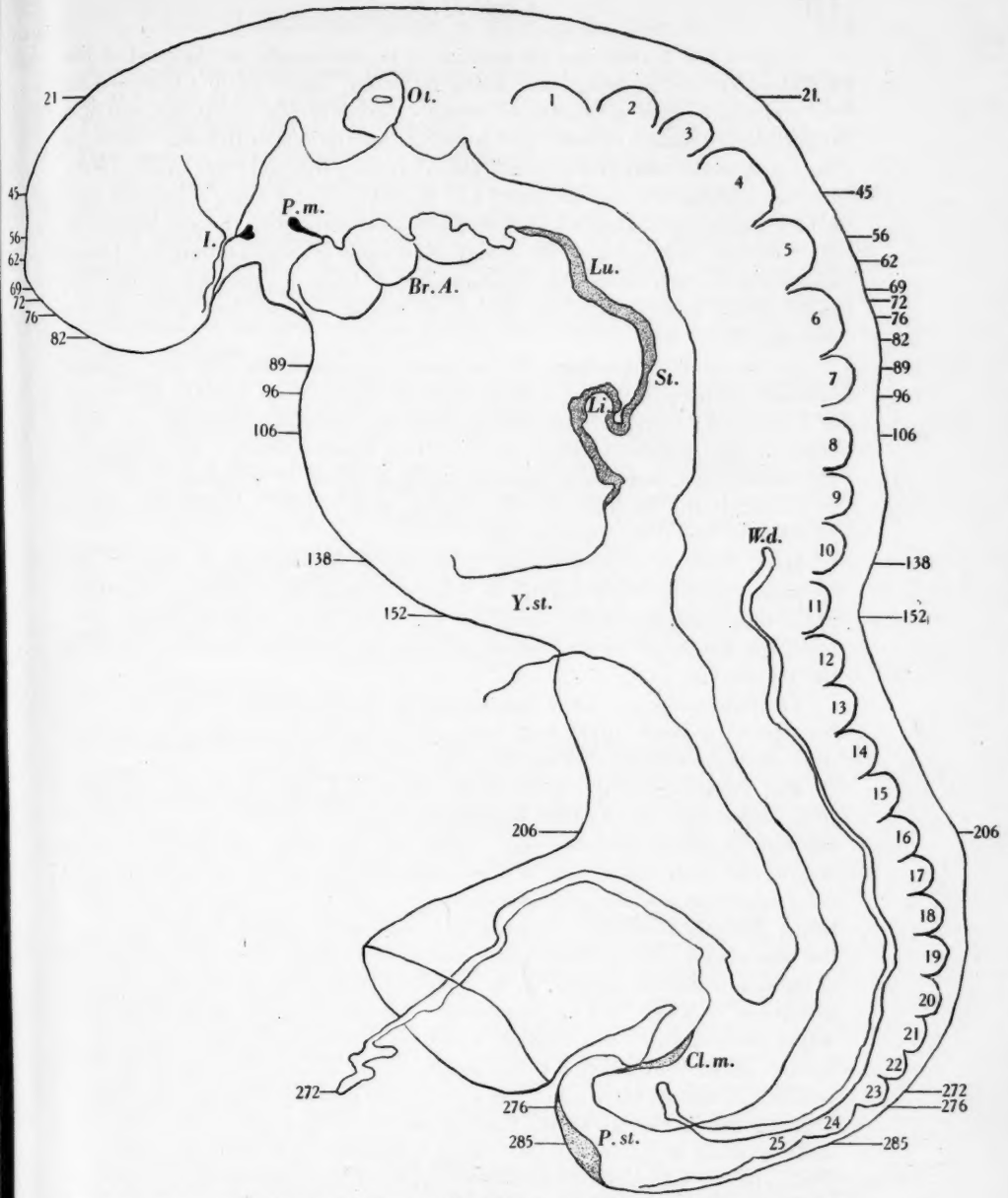
The specimens which this embryo resembles most closely are Thompson's (1907) embryo of 2·5 mm. length and with 23 somites, Politzer & Sternberg's (1930) embryo of 2·7 mm. length and with 25 somites, van den Broek's (1911) embryo A with 28 somites, Broman's (1896) embryo Lf of 3 mm. length and with 30 somites (this last specimen is described as No. 11, in Keibel and Elze's Normentafeln, 1908). The present specimen differs from most others of about the same stage in the greater development of the head, the greater length of the hindbrain, and the more definite neck bend.

SOMITES

There are 25 somites on the left side and 24 on the right, and beyond these, two additional somites are in process of differentiation on each side of the body, but are not isolated from each other (Text-fig. 1).

The 1st somite on each side is indefinite and merges into the general mesenchyme of the head, and it is situated at the level of the summit of the 8th neuromere of the hindbrain. The division of the somites into occipital, cervical, thoracic, etc., has been a matter of some difficulty to me, as it appears to have been to others, for in looking through the literature one finds the number of somites, described as occipital, varying from 2 to 5, and the allocation of certain somites to certain regions seems to have been made without any very obvious criteria having been employed. As long ago as 1901, Bardeen & Lewis pointed out (p. 10) that "the divisions of the myotomes into occipital, cervical, thoracic, lumbar, sacral and coccygeal groups depends upon the nerves and skeletal structures related to the body segments in which the myotomes lie", and "before the spinal nerves have appeared they (the occipital myotomes) cannot with certainty be distinguished"; and (p. 11) "the most caudal myotome lying opposite the arm bud may, therefore, be taken to represent the first thoracic segment". Bartelmez & Evans (1926) have shown that the relative distance between the first pair of somites and the otic plate gradually increases as a result of growth of the intervening region. That there appears to be a caudal migration of the first somite relative to the neuromeres is evident if one notices the position of the first somite in embryos of gradually increasing age, thus:

Embryo	Somites	Neuromeres	Level of first somite
Corner (1929)	10	5	Slightly caudal to 5
Heuser (1930)	14	5	Just caudal to vagus, or 7
Atwell (1930)	17	7	At level of 7
Watt, VI (1915)	17–19	9	Between 7 and 8
Davis (1923)	20	9	Between 7 and 8
Girgis (1926)	22	6	Well caudal of 6
Thompson (1907)	23	7	Just caudal to 7
Johnson (1917)	24	9?	Crest of 8
Present specimen	25	10	Crest of 8
Waterston (1914)	27	7	Well caudal of 7



Text-fig. 1. Reconstruction of embryo from the left side. *I.* infundibular region; *P.m.* pharyngeal membrane; *Ot.* otocyst; *Br.A.* branchial arches; 1 to 25, somites; *Lu.* lung bud; *St.* stomach; *Li.* liver bud; *W.d.* Wolffian duct; *Y.st.* yolk stalk; *Cl.m.* cloacal membrane; *P.st.* primitive streak; the numbered horizontal lines indicate the level of corresponding sections.

Johnson (1917) describes the position of the 1st somite, at the crest of the 8th rhombomere, as being that which he would expect to find occupied by the 2nd somite from his studies of young embryos of pig, sheep, cat, and of a slightly older human embryo; and from the descriptions of Butcher (1929) for the rat, Atwell (1930), Davis (1928), Heuser (1930), Johnson (1917), Low (1908), Politzer (1928*a*), Thompson (1907) and Watt (1915), for man, it appears that (*a*) the 1st somite is always small; (*b*) its cells are sometimes radially arranged; (*c*) it is often rudimentary; (*d*) it may show signs of degeneration; (*e*) it may show evidence of a reversal in development; (*f*) there is a progressive development of the somites from the tail up to the 4th somite and from there a decrease to the 1st.

In the present specimen the 1st somite is small, its cells are radially arranged, it is rudimentary, it shows signs of degeneration, and the 4th somite is the largest; there is, moreover, a tendency towards continuity and similarity of development between the first three somites which is not shared by the remainder, a fact which might be urged in favour of including these three as occipital; the swelling for the forelimb bud is just recognizable, and is found to extend over somites 8–12.

Here, then, is strong evidence in favour of considering, at least in this specimen, the first three somites as being occipital; their common characteristics, their small size, and the relation which they bear to the developing forelimb; the 4th somite in the present specimen is, therefore, considered as being the 1st cervical.

The fullest account of the structure of the somites at about this period has been given by Watt (1915), and, with certain modifications, the present specimen conforms with his description.

The dorsal edge of the cortex of the somite is turned in, to form the myotome, more than the ventral edge; differentiation between sclerotome and myotome is seen best in the more caudal somites, where the sclerotome cells stream out from the somite in a ventromedial direction; one would expect those sclerotomes which are destined to form vertebrae to have an appearance rather different from those which are to contribute towards the formation of the basis cranii, and one finds that in the more cranial somites the ventral edge of the somite and the dorsal edge have become so folded over that the myocoele is reduced to a mere slit (an appearance due partly to the plane of section), which communicates only through a narrow cleft with the main cavity of the somite, whilst ventromedial to the somite cavity are the cells which form the sclerotome, though there is nothing except their position to lead to their identification. It is in the 4th somite that one begins to see a clear indication of the differentiation of the sclerotome, owing, I believe, to the fact that this is the first somite of which the sclerotome comes to embrace the spinal cord, and of which the sclerotome cells spread medialwards as well as ventralwards, whereas in the case of the more cranial somites the presence of the hind brain precludes such a medial extension; but since a single vertebra is derived from parts

of two adjacent sclerotomes, the 4th presumably takes a share in the formation of the basis cranii as well as of the atlas vertebra, which is perhaps what Thompson (1907) had in mind when he stated (p. 164) that the first three, or three and a half, somites might be considered as occipital.

As in Gage's (1905) embryo of 3 weeks, intersomitic grooves on the side of the head are well marked and help in the identification of the first three somites.

An external limiting membrane to the cells of the dermatome is not generally recognizable; but in some sections a separation of the ectoderm from the underlying tissue has occurred and in these sections the outer edge of the dermatome has a very definite and clean cut border which looks very like a limiting membrane.

There is not the same intimate association between the medial surface of the somite and the neural tube as is described by Watt, but the two structures are separated, especially as regards the more anterior somites, by loose mesenchyme, by the migrating cells of the neural crest and by a longitudinal vessel which is specially large in the 10th, 11th and 12th intersegmental regions and which is formed by a union of the dorsal divisions of lateral intersegmental branches of the aorta; more caudally the association between somite and neural tube is closer, since the cells of the neural crest are here interposed between the neural tube and the dorsal angle, rather than the medial surface, of the somite, and the vessel referred to above cannot be traced beyond the 15th somite.

LIMB BUDS

The stage of development of the limb buds resembles closely that of Mall's embryo XII, of which, however, Bardeen & Lewis (1901) state, "There are no external visible signs of limb buds", which was, indeed, my first conclusion in the present case. Politzer & Sternberg (1930) give a figure (9*d*) of a section through an embryo of 2·7 mm. in length, which is very like a section through the arm bud in my embryo.

The forelimb bud is in a very early stage of development, and it shows as a small rounded elevation caudal and dorsal to the prominence of the heart. In the sections, where I was able to recognize it only after prolonged and careful examination, it shows as a thickening of the somatic mesoderm, opposite about the 8th to the 12th somites and just to the lateral side of the dorsal angle of the embryonic coelom, but no definite boundaries can be given for its extent. It is recognizable by the following characteristics: the mesoderm is thickened; the cells of which it is composed resemble those of a sclerotome, having clear-cut rounded nuclei rather than the irregular and more indefinite nuclei of the mesenchyme cells, and being deeply stained; the cells are packed more closely than those in the loose mesenchyme; there is a definite thickening of the ectoderm over the limb bud swelling, as described by Gage (1905) in a specimen which showed the limb buds in a more advanced stage; there is an abrupt line of demarcation between the cells of the limb bud and those of the

somite, even though, at the widest part of the somite, the two structures are in close apposition.

With regard to a contribution from cells of the myotome towards the limb bud, there is a close resemblance between this specimen and embryo XII, 2·1 mm. in length, belonging to Dr Mall's collection; of this embryo W. H. Lewis (1902) states (p. 149): "The origin of the cells which cause this swelling I am not able to determine, though there are suspicious looking processes from the myotome". Zechel (1924), referring to Grosser's embryo Kl 32, of 3·4 mm. length, writes of an outwandering of cells from the lateral plate of the myotome into the anlage of the upper limb.

Lewis, in spite of the "suspicious-looking processes", shows, in his Fig. 2, a quite definite separation between "arm bud" and "8th cervical muscle plate"; this is just what I find in the present specimen, and I have the impression that there is, in this specimen, no contribution from the myotome towards the limb bud.

The posterior limb bud is even more difficult to identify than the anterior; there is a symmetrical swelling on each side of the body at the level of the last two somites (lumbar 1 and 2) and it extends over the succeeding unsegmented mesoderm. This swelling is recognizable in the photographs (Pl. I, figs. 1 and 2) and is, presumably, the Wolffian ridge; there is nothing particularly characteristic about any part of it by which it may be identified as a limb bud, but on the other hand it does appear to have a definite relationship to the Wolffian duct, for at this point the duct comes close to the surface and its course is exactly marked out by the groove which forms the ventral limit of the swelling; Bremer (1906) shows a rather similar swelling in his 4 mm. embryo, but states (p. 459), "There is no trace of posterior limb", in his figures the Wolffian duct shows a similar relationship to the swelling to what it does in my specimen.

ALIMENTARY TRACT

The pharynx is open anteriorly, for the pharyngeal membrane has ruptured and its remains can be seen (Text-fig. 1) adhering to the ventral and dorsal walls. There are three pharyngeal pouches on each side, all of which reach the ectoderm, and there are three branchial grooves on the exterior.

The first two pouches on each side have well developed dorsal diverticula and much smaller ventral diverticula.

The pharynx, as a whole (Text-fig. 2), is concave dorsally from side to side on its dorsal surface and concave ventrally on its ventral surface.

The floor is marked in its most anterior part by a deep median groove, and a much shallower groove likewise marks the dorsal wall, or roof. At the point of attachment of the pharyngeal membrane the groove on the roof of the pharynx is continued on to the roof of the mouth, and there it divides into two limbs with a rounded ridge between them; this ridge is due to the downgrowth of the forebrain and emphasises the close relationship between brain and pharynx at this point; just to the cranial side of the pharyngeal membrane the pouch of

Rathke shows as a small diverticulum of the mouth cavity; Seessel's pouch cannot be identified.

The deep median groove on the floor of the pharynx also is prolonged forwards to the mouth; it is from this groove that one would expect to find the outgrowth of the median thyroid rudiment, and there is to be seen, in four sections, just at the junction of the first and second branchial arches, a small outgrowth from the pharyngeal floor towards the ventral aorta; I believe that this is the median thyroid rudiment, though it is smaller than in other embryos of about the same age.

In the section just before the deepest part of the outgrowth of the thyroid there is the beginning of a very curious structure which consists of a hollow tubular evagination of the pharyngeal floor projecting freely into the lumen of the pharynx in a cranial direction and covering a distance of seven sections.

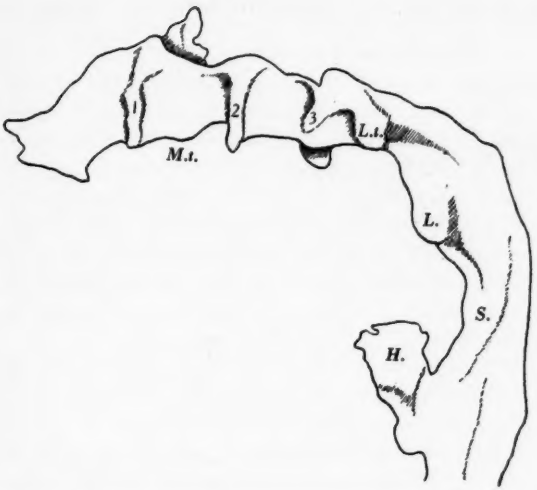
This structure begins on the pharyngeal floor as a slight ridge which then becomes pinched off from the floor so that it becomes a tube lying free in the lumen of the pharynx.

I thought at first that it might be an internal gill filament. Since Grosser (1912) first described them, such structures have been seen in embryos of about this age, and younger, by Heuser (1930), Atwell (1930), Veit & Esch (1922), Politzer (1928*a*), Davis (1923), and Girgis (1926). In all these cases, however, there are merely proliferations or ridges of the entoderm and not long, freely projecting structures such as I have described; furthermore, the correctness of Grosser's interpretation has been questioned by Boyden (1918), who considered that the structures described by the above observers are more comparable with the outpocketings which occur in the pre-auditory region of the chick, and that they probably are not rudimentary internal gills (a comment with which Heuser appears to agree) for the following reasons: gills are essentially ectodermal derivatives; gills are least frequently developed in association with the first pouches in water breathing vertebrates; the embryonic stages at which these structures have been observed are too early, for all the pouches have not yet reached the ectoderm. The truth of the statement that gills are essentially ectodermal derivatives is, I believe, still open to question. These rudimentary structures (they are not present in my embryo) seem always to grow from the ventral wall, and always occur near the closing membrane of the first pouch; the structure that I am describing in the present embryo grows from the ventral wall also, but is attached close to the middle line and as far away as it can be from the first closing membrane; it thus seems very unlikely that it is a rudimentary internal gill, though it does appear as if, during life, it had been waving about freely in the pharyngeal lumen—an ideal position for a gill filament—but there is no evidence of blood cells in its lumen but only a few mesoderm cells. Its close relationship to the thyroid led me to consider the possibility of its association with an endostyle, but this, I believe, is out of the question; in short, I am unable to offer any interpretation of this structure.

Attention has been called to the small size of the median thyroid rudiment; the lateral rudiments, on the other hand, show very well, and the condition is very like that described and figured by Weller (1933), in an embryo 4 mm. long. It was not until I had read Weller's paper that I realized the significance of what I at first took to be 4th pouches, though I had been surprised at their size compared with the 3rd.

The lateral thyroid rudiment shows (Text-fig. 2) as a rather large outgrowth from the pharynx just to the caudal side of the small 3rd pouch; the cavity of the pharynx is continued into the outgrowth and round its margin the epithelium is thickened. It may be possible that the small size of the median rudiment is correlated with the greater development of the lateral rudiment.

The part of the alimentary tract that succeeds the pharynx has been a source of difficulty in interpretation to several observers; in the present specimen it shows, at first, a slit-like lumen and walls of uniform thickness, but, after eight sections, there appears a rather spherical outgrowth on the right side (Text-fig. 3*a*) and, after a few more sections, there is a corresponding dilatation on the left; the difference in level of the two outgrowths is due to a slight obliquity of the sections through this part of the gut. These two outgrowths, which I will call the cranial swellings, arise from the ventral part of the gut, whereas the dorsal part of the gut has a slit-like lumen and is separated from the ventral expanded part by a slight constriction; beyond this cranial swelling the gut shows a lumen which is suddenly much reduced in ventro-dorsal diameter, being the continuation merely of the slit-like dorsal part of the more cranial portion of the gut; farther on the gut has a pear-shaped lumen, wide ventrally where it forms what I will call the caudal swelling (Text-fig. 3*d*), and slit-like dorsally, and it becomes continuous, in the septum transversum with the part of the gut from which the liver arises (Text-fig. 3*f*). The cranial swelling shows a slight indication of division into two; its walls are much thickened; it is to the cranial side of the septum transversum; it lies dorsal to the sinus venosus and atrium, just where these two cavities communicate; the swelling is greater towards the left side than to the right, and on the left side it grows towards the pericardial cavity and on the right towards the pleural passage. The caudal swelling is much smaller; it shows no evidence of duplicity; it is close to the hepatic diverticulum, and it shows no thickening of the ventral wall, which is, indeed, rather thinner than the dorsal wall. I show (Text-fig. 3) a series of outlines of sections of the gut at different levels, which may be compared with similar figures by van den Broek, and by Girgis and others, and from which it will be seen that I am in disagreement with van den Broek and Girgis, both of whom show what they describe as lung, in the same section as the liver. In Text-fig. 4 I show a drawing of this region in the present specimen, together with tracings of the same area in the embryos described by Corner (1929), Heuser (1930), Low (1908), Davis (1923), Thompson (1907, 1908), Girgis (1926), Johnson (1917), and Waterston (1914). In the earlier stages (Corner and Heuser) lung and liver are very close to each other; in Low's

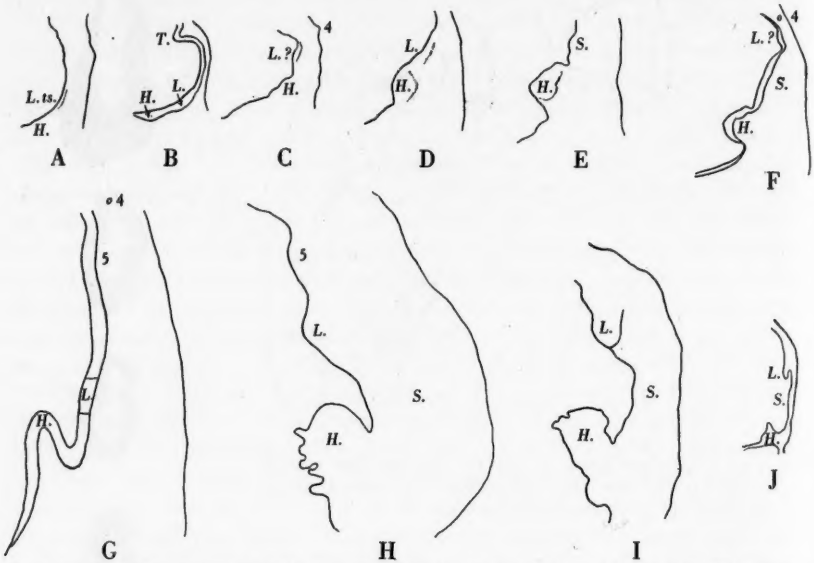


Text-fig. 2. Reconstruction of pharynx and foregut. 1, 2, 3. pharyngeal pouches; *M.t.* median thyroid; *L.t.* lateral thyroid; *L.* lung bud; *S.* stomach; *H.* liver bud.

Text-fig. 3.

Text-fig. 3. Camera lucida drawings of outlines of the gut at different levels. *a*, section 62; *b*, section 69; *c*, section 76; *d*, section 82; *e*, section 89; *f*, section 96. Compare with Text-fig. 1.

specimen I have marked a doubtful swelling which I believe represents the lung bud, but from his statements I do not think that this is the groove which Low considers (p. 245) "may indicate the lung groove", but that he would place it some distance further cranialwards. In Davis' specimen liver and lung still are in close apposition, but in the embryo described by Thompson there is a sudden increase in the distance between the two rudiments, but what Thompson interprets as stomach Grosser (1912) would call lung bud, and Davis after a study of his own specimen naturally takes the same view as Grosser; Girgis, in a 22-somite embryo, and van den Broek, in one of 28 somites,



Text-fig. 4. Outline drawings of the foregut region in different embryos. A, Corner; B, Heuser; C, Low; D, Davis; E and F, Thompson; G, Girgis; H, Johnson; I, present specimen; J, Waterston; *L.ts.* laryngo-tracheal sulcus; *H.* liver bud; *T.* median thyroid; *L.?*, doubtful lung rudiment; *L.* lung bud; *S.* stomach; 4 and 5, doubtful fourth and fifth pharyngeal pouches.

show the lung and liver rudiments so close to each other that parts of both are cut in the same section, a fact which is in keeping with Grosser's interpretation of Thompson's specimen; in Johnson's specimen, of 24 somites and in the present one, of 25 somites, there is a much larger interval between lung and liver, and I agree with the interpretation of Johnson, as far as these two rudiments are concerned, though I am not so certain about his ?5th pharyngeal pouch; in Waterston's embryo, of 27 somites, there is again a wide interval between lung and liver, and he shows a lung bud which can surely be interpreted as nothing but lung. In the earlier stages to which I have referred the

lung bud is shown as being bifid at its caudal end, but such a bifid condition is not present in the more caudal swelling of Thompson's embryo, whereas it is present in the cranial swelling which Thompson calls lung bud. Furthermore, if Grosser is correct in his interpretation of the more caudal swelling as lung bud, it would mean that this structure would come to an end right up against the dorsal surface of the liver bud, behind which it is hidden. The resemblance between Thompson's specimen and my own is so close that I find it difficult to believe that Grosser's interpretation is correct.

In some of the figures referred to above (C, F, G, H) the positions of the 4th pharyngeal pouches and of doubtful 5th pouches are indicated; in Fig. F, Grosser would interpret what I have labelled L? as 5th pouch?. In view of Weller's (1933) work, I am a little doubtful as to the correctness even of the designation of certain of the structures interpreted as 4th pouches, and more so still with regard to the doubtful 5th pouches; for examples I refer to the figures of Low, Thompson, Girgis, Johnson and Davis, though, owing to the absence of figures of the whole pharynx, it is not possible to be certain as to the correctness of their interpretation.

In the present specimen the 3rd pouch is quite small and I do not believe that what I have called lung bud could be interpreted as 5th pouch, especially as I have not been able to recognize a 4th pouch.

It would thus appear that after the 20-somite stage, as exemplified by Davis' specimen, there is a rapid growth of the foregut, and liver and lung thus come to be widely separated from each other; such growth may have an influence in obliterating the dorsal concavity of the body of the embryo by allowing more room for the developing heart, and it has been noticed that this concavity does become less in later stages.

LIVER

The part of the gut which succeeds the stomach shows an increase in its ventro-dorsal diameter and from it the liver arises; the liver diverticulum grows from the gut at first ventralwards and then towards the left, so that the lumen of gut and of liver diverticulum together form an L-shaped cavity; the liver then grows cranialwards and to the right, caudalwards and to the left and thus, as shown also by Thompson (1908), the liver as a whole lies obliquely across the ventral surface of the gut (Text-fig. 5).

Two very obviously different parts of the liver outgrowth can be recognized well in a model (Text-figs. 1 and 5); a large cranial portion with uneven surface extending on each side of the gut, and a small smooth caudal portion confined to the ventral surface of the gut and not extending in a lateral direction; the uneven surface of the cranial portion is due to the outgrowth of liver cords from it, and it is the "pars hepatica", of Maurer, of the diverticulum; the smoothness of the smaller caudal portion is due to the absence of liver cords, and it is the "pars cystica" of the liver outgrowth.

Liver cords grow out from the pars hepatica in a cranial and in a ventral

direction, to the right and to the left, forming in all about twenty-five main stems, of which most grow ventralwards, about an equal number to the right and to the left, and least of all in a cranial direction.

In the septum transversum the distinction between the cells of the liver cords and of the mesenchyme is quite clear, the liver cords being recognized by the large size of their cells, and, as van den Broek (1911) pointed out, by their being more darkly stained, and by their being more closely packed together than the mesenchyme cells. All the liver cords are solid outgrowths from the walls of the diverticulum and there is not any extension into them from the adjacent cavity of the gut.

Text-fig. 5. Reconstruction of lung and liver from in front. *P. hep.* pars hepatica; *P. cyst.* pars cystica, of liver bud.

Bremer (1906), in his description of an embryo of 4 mm., called attention to the relationship between the developing liver and the vessels in the septum transversum, and expressed the opinion that the branching of the vitelline veins was independent of the liver cords, and that the ultimate close relationship of the two structures was the inevitable result of their mutual growth. The same conditions are found in the present specimen, in which it is only at the cranial part of the liver diverticulum that vitelline and umbilical veins join, so that it is to the vitelline veins that the liver is most closely related. Without giving a tedious description of the branching of the vitelline veins, it may be said that there is one main vessel on each side which passes through the septum transversum, and that though bits of the vessel may get pinched off, such pinching off appears to be due to mesenchymal rather than to hepatic invasion, and the end result is that there is formed a complicated network of vessels, many of which come to be blood spaces completely isolated from each other and surrounded with liver cords; yet it would be untrue, I believe, to describe the liver cords as breaking up the veins, and I would rather say that though by their mutual growth liver cords and vessels do come into close contact, the cords are not the principal factor in the branching of the veins, though they may by their pressure encourage such branching.

YOLK SAC AND HINDGUT

Caudal to the liver bud the ventrodorsal diameter of the gut becomes reduced and, as the opening into the yolk sac is approached, it is restricted also from side to side; the wall of the gut remains thick in its dorsal part but ventralwards becomes much thinner, and it is this thin portion which is continued into the yolk sac, so that there is a very striking difference between the

thick dorsal part of the gut on the one hand and the thin ventral part and yolk sac wall on the other. This is similar to the condition described by Johnson (1917), but different from that illustrated by Davis (1923) and Politzer (1928 *a*).

The yolk stalk can be seen very well in the photographs, leading out from the widely open ventral body wall; the yolk sac has collapsed on three sides, and the blood islands in its wall make knob-like projections on its surface. Caudal to the opening of the yolk sac the cavity of the gut becomes gradually reduced but retains a pear-shaped outline with the wider end directed ventralwards, until, after about 75 sections, it opens into the cloaca.

CLOACA AND ALLANTOIS

The cloaca, with the allantois arising from its cranial and ventral part, is shown in Fig. 1. The allantois follows the course of the body stalk, passing at first cranialwards and then turning caudalwards; it thus presents a curve convex in a cranial direction, with ventral and dorsal limbs, and its total length is 1·45 mm., which is rather more than is found in other embryos of a similar stage of development.

The calibre of the allantois is nearly uniform throughout, but the ventral, or caudal limb, is constricted here and there, and at its termination is considerably widened and bent upon itself in an **S**-shaped manner.

A lumen is present throughout, wide at the beginning of the duct, then gradually decreasing towards the end, where it becomes dilated again, the **S**-shaped portion having the greatest diameter of the whole duct.

The concavity of the allantoic loop is occupied by the great blood lake formed by the fusion of the umbilical veins in the body stalk; only one anastomosis between the two umbilical arteries is present, it occurs only a short distance before the end of the allantois and it does not appear to have any constricting effect on the duct, as Atwell (1930) suggested might be the case.

In the body stalk, considerably ventral and slightly to the right of the allantois, there is a curious structure (Pl. I, fig. 3) resembling the "Epithelbläschen" described by Veit & Esch (1922) in an embryo of the 4th week, with 8 pairs of somites; it is a closed cylindrical sac, like a portion of the allantois in appearance and, like the allantois, is surrounded by a clear space which serves to distinguish it at once from a blood vessel. This vesicle does not appear to have been constricted off from the main part of the allantois, as Davis (1923) suggested might have been the case in the specimen described by Veit & Esch, but it is a completely isolated structure; its wall is one cell thick, its cavity is empty, except for a little epithelial debris, and it tapers rather to a point at each end, which is the one feature in favour of its having been constricted off from the allantois.

The cloaca is expanded ventrodorsally and flattened from side to side; it is continuous caudally with the tail gut, which itself extends to within three sections of the tip of the definitive tail. From the caudal margin of the body stalk to the beginning of the tail the ventral body wall of the embryo is marked

by a median groove which gradually deepens as it is traced in a caudal direction; throughout the length of this groove ectoderm and entoderm are in contact, at least there is no mesoderm intervening, though the entoderm is drawn ventralwards in a thread-like manner so that, towards the caudal end, the connexion between entoderm and ectoderm is very slender; this area of contact is the cloacal membrane (Text-fig. 1).

The sides of the cloaca are marked, nearer the dorsal than the ventral border, by a groove which runs parallel with the dorsal edge of the gut and which is continued round the curve of the cloaca towards the tail; this groove indicates the future subdivision of the cloaca into rectum and urogenital sinus. Of these two parts of the cloaca the ventral urogenital sinus is by far the larger, and is expanded from side to side in contrast with the laterally compressed rectum. Watt (1915), in his description of twin embryos of 17–19 somites, referred to the impending subdivision of the cloaca and to a groove similar to that to which I have called attention; he showed, in his Fig. 7*c*, of embryo V, in contrast with the present specimen, a wide dorsal "rectal bay" and a laterally compressed ventral "bladder bay", whilst his embryo VI, shown in Fig. 7*b*, is much more like the present specimen.

From a study of this embryo I have the impression that the dorsal rectal portion of the cloaca is carried caudalwards, caudal to the bladder portion and is then continued ventral to the urogenital sinus and so into the tail, so that a section through the cloaca near the tip of the tail shows a dorsal laterally compressed portion, which is the rectum, an intermediate expanded portion, which is the urogenital sinus, and a ventral compressed portion, which is the caudal part of the rectum, or the tail gut; with the straightening out of the tail and the atrophy of the tail gut, the rectum will be left opening dorsally and the urogenital sinus ventrally, after the rupture of the cloacal membrane.

UROGENITAL SYSTEM

This is composed of a few isolated pronephric rudiments and of a series of mesonephric tubules, or vesicles, which are connected with each other through a longitudinal mesonephric duct that runs caudalwards and just fails to open into the cloaca (Text-fig. 6).

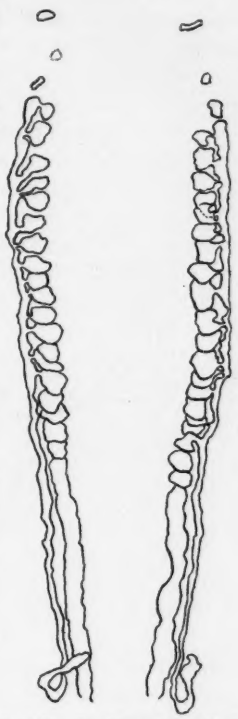
The pronephric rudiments are three on each side.

On the left side the first indication of a pronephric tubule occurs as a thickening and invagination of the coelomic epithelium on a level with the middle of the hepatic diverticulum and with about the middle of the 8th somite; the invagination is directed at first dorsalwards and then makes connexion with a small, blind and almost solid, though still hollow, vesicle. The second rudiment is much smaller; it is placed opposite the caudal half of the 9th somite, caudal to the hepatic diverticulum, and it arises in the same manner as the first, but the invagination is directed lateralwards rather than dorsalwards and ends in a small, solid clump of cells, the whole structure being much

smaller than the first and not extending so far in a dorsal direction. The third rudiment is larger than either the first or second; it is related to about the middle of the 10th somite, and is essentially similar to the first and second tubules and ends in a definitely hollow vesicle.

The next tubule, which begins towards the caudal part of the 10th somite, is the first which joins the Wolffian duct; it arises, like the previous three tubules, by a connexion with the coelomic epithelium and then turns in a caudal and dorsal direction to join the Wolffian duct, which has a blind cranial end beginning at almost the same level as the tubule.

There next occurs a series of 20 vesicles, or tubules, which appear to arise *in situ*, though in nearly every case there can be recognized the remains of the nephrogenic cord through which they were connected originally with the coelomic epithelium; all of these vesicles are quite separate from each other, all possess a lumen, and all, except the last two and Nos. 15 and 17, make connexion with the Wolffian duct. A good guide to the point of fusion of vesicle and duct is found in the presence of a clear space, which surrounds vesicle and duct individually where these two structures are separate (Pl. I, fig. 5), but which becomes common to both duct and vesicle where these are united; such spaces are of common occurrence in human embryos, and it has been suggested by Prof. Evatt, in verbal communications to the Anatomical Society, that they are early lymphatic spaces. Caudal to the 20th vesicle there is a nodular cord of mesoderm which fades away towards the tail and shows a series of nine or ten dilatations, the beginnings of new vesicles.



Text-fig. 6. Reconstruction of nephric system from in front.

On the right side the first pronephric rudiment arises in a manner similar to that on the left side, there being a thickening of the coelomic epithelium, an invagination, a dorsolateral and caudal extension terminating in a blind and hollow vesicle.

The first pronephric tubule lies at the level of the cranial part of the 8th somite. At the extreme caudal end of this somite there is a definite thickening of the coelomic epithelium, and in the mesenchyme a few cells which may represent a degenerated tubule. The third rudiment is similar to the first and is related to the cranial part of the 9th somite; it is succeeded, at the caudal end of this somite, by a tubule which, as on the left side, turns caudalwards to join a well-defined duct. Beyond this point there is a series of 19 tubules, or vesicles, all of which show a lumen, and all, with the exception of the last four

and No. 13, are connected with the Wolffian duct. As on the left side, the last tubule is followed by a nodular cord which has about ten dilatations.

The Wolffian duct begins in the manner already described, opposite the 10th somite on the left side and opposite the 9th somite on the right. A lumen can be traced throughout its entire length, being especially large towards the cranial end of the duct and frequently showing enlargements after the union with it of a mesonephric vesicle. At its caudal end the duct is bent, in conformity with the curvature of the tail region of the embryo, and ends blindly close to the cloaca; the duct has a blunt and rather small and tapering end, but just before its termination it is much larger and it is at this point that it attains its greatest diameter; where the duct turns round, following the curvature of the tail, it is compressed from side to side and is much smaller than it is just before its termination.

The Wolffian duct comes to an end before reaching the cloaca, and close under the ectoderm; yet the relationship between ectoderm and duct is not so

Text-fig. 7. Camera lucida drawings of last eleven sections through Wolffian duct, being sections 279–269, from left to right.

close at the very end of the duct as it is some distance further cranialwards; I show (Text-fig. 7) camera lucida drawings of the last 11 sections of the duct and adjacent ectoderm; at about the bend of the duct or slightly to the caudal side of this, the duct is so close to the ectoderm that I was at first under the impression that an actual opening of the duct on to the surface was present at this point, especially as the ectoderm here is rather irregular; such an opening, however, does not exist, and it is only that there is very close relationship and, indeed, actual contact between the duct and the ectoderm, as may be seen in the figure.

It thus appears that in this embryo there is no contribution of the ectoderm towards the formation of the duct.

The course of the more caudal part of the Wolffian duct can be marked out almost exactly on the surface of the embryo, for the caudal part of the coelom, or inferior peritoneal canals, underlies a prominence—the Wolffian ridge—on each side of the body; this prominence is separated from the more ventral part of the body of the embryo by a groove which turns forwards towards the tail, and at the bottom of this groove, which can be seen in the photograph of the intact embryo, there lies the caudal part of the duct; this is just like the condition described by Bremer (1906) in an embryo of 4 mm. length, in which the Wolffian duct failed to reach the cloaca on one side.

The pronephric tubules have been described above as arising from an in-

vagination of the coelomic epithelium, whereas the mesonephric tubules appear to arise *in situ*; there is, however, on the left side at about the level of the middle of the third mesonephric vesicle, a very well-marked nephrostome (Pl. I, fig. 5); it is a deep and wide invagination of the coelomic epithelium and is directed medialwards and dorsalwards; it has no connexion with the vesicle but lies ventral to it. In association with the first mesonephric vesicle on the right side there is a less well marked, but still deep, invagination of the epithelium, which I believe to be a nephrostome; these are the only nephrostomes present, though nearly all the vesicles have a connexion with the coelomic epithelium through the remains of the nephrogenic cord. When, in a reconstruction, the dorsal wall of the body cavity is viewed from the front the line of connexion, through the nephrogenic cord, between nephric rudiments and the coelomic epithelium is found not to be straight but to approach the middle line as it is traced in a caudal direction, and it shows an abrupt medial bend at the point of transition from pronephric vesicles to mesonephric vesicles. Watt (1915) has referred to the thickening of the coelomic epithelium along the mesentery and to the medial side of the urogenital fold; a urogenital fold is not well marked in this specimen, though there is a clear indication of it in the pronephric region, where it is limited medially by the nephrostomes of the pronephric tubules; in the mesonephric region the vesicles by their size cause an elevation of the dorsal wall of the coelom, and this may be regarded as the urogenital fold; it is the epithelium of the dorsal wall of the coelom that is so characteristic in the mesonephric area, and the arrangement of the cells is so definite that it may almost be employed as a means of differentiating the mesonephric region from the pronephric; over the mesonephric rudiments the epithelium forms only a thin layer of cells arranged with their long axes parallel to the surface, but just where the remains of the nephrogenic cord are connected with the epithelium there is a sudden and very striking change, the cells here being arranged with their long axes at right angles to the surface and thus seeming to form a much thicker layer than over the vesicles; this arrangement of the cells at right angles to the surface is continued from the nephrogenic cord along the sides of the mesentery; in the pronephric region all the cells of the dorsal wall of the coelom appear to be arranged at right angles to the surface, and seem merely to be smaller over the pronephric rudiments than in the adjacent epithelium. There are no glomeruli present.

The arrangement of the nephric system in this embryo corresponds very much with that described for other specimens of about the same age, for example, MacCallum's (1902) of 19 somites, Davis' (1923) of 20 somites, Girgis' (1926) of 22 somites, Thompson's (1907) of 23 somites, and Johnson's (1917) of 24 somites; but Davis and Girgis consider that the pronephros extends as far as the 12th (Davis) and the 13th (Girgis) somites; in the present specimen I believe that the pronephros does not extend beyond the level of 10th somite, because on each side the first three tubules, which I have called pronephric, form an isolated group, they are all rudimentary, and they all

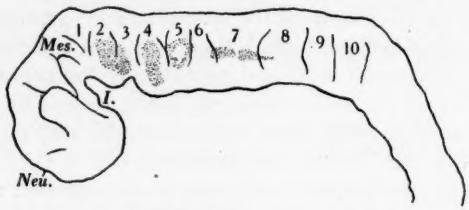
appear to arise in a similar manner by an invagination of the coelomic epithelium, whilst those vesicles, which I have called mesonephric, form a continuous series, they are all well developed, they all seem to have arisen *in situ*, and they all are alike in appearance.

NERVOUS SYSTEM

The brain and spinal cord form an almost completely closed tube, the anterior neuropore being closed and the posterior being just in the process of closing.

There are three brain vesicles, and in the following account they are described as being in the position shown in the figures.

Forebrain. This is a globular mass which overhangs the pharynx and mouth region; at the point indicated in Text-fig. 8 there can be seen the remains of the anterior neuropore, the closure of which has been just completed, and the rolled-in edges of the brain wall show very well in the sections and it can be



Text-fig. 8. Reconstruction of brain. *Neu.* anterior neuropore; *I.* infundibular region; *Mes.* midbrain; 1–10, neuromeres; stippled areas from left to right: trigeminal ganglion; acousticofacial ganglion; otocyst; glossopharyngeal and vagus ganglia.

seen that the head ectoderm also is tucked a little into the groove at the point of closure; the condition is very like that figured and described by Sternberg (1927) in an embryo of 25 somites, and 2·7 mm. in length. Just to the cranial side of the pharyngeal membrane there is a slight downgrowth of the floor of the brain which marks the infundibular region (Text-figs. 1 and 8), and at this point the brain wall and mouth cavity are in contact and it is here that the notochord comes to an end.

From the sides of the forebrain there arise the optic vesicles, but there is no evidence of a lens thickening in the overlying ectoderm.

With regard to the markings on the surface of the brain interpretation is not easy, for there has been an undoubted shrinkage of the brain, a shrinkage which appears to involve chiefly the ependymal and mantle zones, whilst the marginal zone does not seem to be much altered. Bearing this fact in mind, it may be stated that the forebrain, between the main mass of the optic vesicles, is constricted from side to side, whilst on a level with the tip of the vesicles it becomes expanded. At this level a groove is seen on the roof, when the brain is viewed in profile, and it is a similar groove that Wen (1928) showed in his Fig.

7*c*, of a 22-somite embryo, and which he considered as marking the separation between forebrain and midbrain. The side of the brain just behind this point is marked by two grooves which together form a V with apex directed towards the infundibular region; Wen showed similar grooves in his 22-somite specimen, and he regarded the more posterior as dividing the midbrain into anterior and posterior parts, and the more anterior as marking the separation on the side of the head between forebrain and midbrain. Johnson (1917), in his specimen of 24 somites, shows a groove corresponding with the posterior limb of the V-shaped groove and he considers that it is this groove which separates forebrain and midbrain, whereas Wen regards this groove as dividing the midbrain into two parts; in this I am in agreement with Johnson, as far as my embryo is concerned, that is to say I believe that the posterior limb of the V-shaped groove marks the separation between forebrain and midbrain; the anterior limb of this groove I believe to be due to the overhanging growth, in front of it, of a developing cerebral hemisphere, such as Watt (1915) shows in his embryo VI. An oblique groove marks clearly the separation between midbrain and hindbrain, the latter being recognized by its neuromeres.

The midbrain is the smallest of the three segments, and here the forebrain and hindbrain are bent so as to form a right angle with each other.

The hindbrain is the largest segment. The roof already shows the thinning of the future roof of the fourth ventricle, and when the embryo was in alcohol it was possible to see, through this thin roof, a series of alternating expansions and constrictions—the neuromeres.

The neuromeres resemble closely those described by other observers, but they show more clearly than in other specimens (Text-fig. 9); there are ten of these segments which involve the lateral wall of the brain. The 2nd, 4th, 6th and 7th involve also the floor, to which they give an irregular outline; it is the grooves between the projections of the floor caused by these neuromeres, and especially the 4th, which are referred to by Thompson (1907), Bremer (1906), and van den Broek (1911), as having given rise to a difficulty in their interpretation, and van den Broek suggested that they might have some association with the Vth and VIIIth ganglia, which is indirectly true in view of the relation of these ganglia to the neuromeres which are responsible for the production of these grooves. The ganglion of the trigeminal nerve lies lateral to segments 2 and 3; the acoustico-facial ganglion is lateral to segment 4; the otocyst is lateral to segment 5; the glossopharyngeal ganglion is lateral to segment 7, and the vagus ganglion is lateral to segments 7 and 8 (Text-figs. 8 and 9), but the glossopharyngeal and vagus ganglia are difficult to define and appear only as rather diffuse clumps of cells. The first somite is opposite the 8th neuromere, the 2nd somite is opposite the 9th neuromere, and the 3rd is opposite the 9th and 10th neuromeres and the intervening groove.

Spinal cord. The more cranial part of the spinal cord shows three faintly marked segments, succeeding those of the hind brain and, like them, having no segmental relationship to the somites. In its cranial two-thirds the lateral

surface of the spinal cord is marked by a longitudinal groove corresponding in position with that of the future sulcus limitans on the inside; the diameter of the spinal cord is reduced in its middle part just beyond the dorsal concavity of the body, and it then increases, especially in a ventrodorsal direction, towards the tail; it ends, 30 sections before reaching the tip of the tail, in close apposition to the primitive streak; it is here that the posterior neuropore can be seen just in the act of closing, the condition being essentially similar to what is found at the anterior end of the brain only showing an earlier stage in the process.

Otocyst. This is a hollow, oval vesicle, measuring 0·18 mm. in a craniocaudal direction, and 0·14 mm. from side to side; it is thus of much the same size as the otocyst of embryos described by Davis (1923), Wact (1915), and van den Broek (1911). The sac is open on to the exterior (Text-fig. 9) through an orifice that measures 0·02 mm. in diameter, and which is, as in van den Broek's specimen, nearer the cranial pole than the caudal pole of the vesicle. From the ventromedial surface of the sac there projects forwards a hollow diverticulum for a distance of 0·04 mm. Waterston (1914) shows a rather similar diverticulum, but in his specimen the projection is in a dorsomedial direction. The picture presented by my specimen is very like Arey's (1930) Fig. 488, of a section through a 6-mm. pig embryo, and he refers (p. 492) to the ventromedial diverticulum as "a median outpocketing where the endolymph duct attaches"; yet though the vesicle in this pig embryo and in my specimen are so alike, mine must be much the younger, and it is still open to the outside, and it is not till after the closure of the opening that there arises, from the point of closure, the endolymph duct which then shifts in a ventromedial direction, so that however tempting it may be to interpret it otherwise, it is probable that this diverticulum is a folding due to shrinkage. The vesicle as a whole overlies the 5th neuromere and corresponds almost exactly with it in size; the diverticulum referred to projects towards the groove between the 4th and 5th neuromeres and towards the acoustico-facial ganglion; at the more cranial part of the vesicle, at the level of its opening to the exterior, the relationship between the vesicle and the acoustico-facial ganglion is a very close one, the ganglion appearing to be moulded round the ventral surface of the vesicle. The structure of the vesicle is essentially similar to that of the adjacent wall of the hindbrain and it shows the same arrangement of mantle and marginal zones.

Text-fig. 9. Drawing from photograph of section 21, to show neuromeres, and otocyst. Stippled areas are V, VII–VIII, IX, X ganglia; and somites 1 and 2.

Neural crest. At the level of the 5th somite a neural crest can be recognized

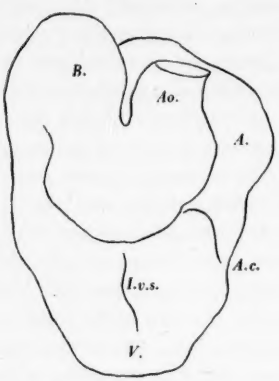
with certainty; from the 5th to the 2nd somite there is a collection of darkly stained nerve cells occupying the position of a neural crest, but they cannot be differentiated clearly from the surrounding mesenchyme, nor do they seem to have any connexion with the cells of the vagus ganglion. A definite neural crest can be traced from the 5th to the 21st somite; it shows no point of maximum development, such as Wen (1928) suggested occurring at the level of the 5th somite in his 17-somite embryo, but from about the 9th or 10th somite there is a gradual reduction in the development of the crest when it is traced towards either the head or the tail.

With regard to the cranial nerve ganglia, the trigeminal and acoustico-facial ganglia are large and well defined, but the glossopharyngeal and vagus ganglia are not seen so clearly. The ganglia, especially the trigeminal and acoustico-facial, in the more cranial sections, i.e. nearer the roof of the brain, appear as circular masses of cells lying close to the marginal zone of the neural tube, but when traced in a caudal direction there is a migration of the cells in a lateral direction, whereby the cells come to lie close up against the ectoderm of the head, which is much thickened at the site of contact of the ganglionic cells. Such ectodermal thickenings are developed in association with each of the four ganglia, trigeminal, acoustico-facial, glossopharyngeal and vagus, and they involve the whole of the 1st, 2nd and 3rd branchial arches with which the ganglia are associated, and the thickening is prolonged in a caudal direction beyond the 3rd arch on to the cardiac area. In connexion with these ganglia, reference may be made to the arrangement of the cells in the walls of the neuromeres; showing particularly well in the case of the 4th neuromere, there is a great thickening of the brain wall at each of the neuromeres, whilst between the neuromeres the wall is much thinner; such thickenings of the brain wall in the region of a neuromere are suggestive of there being an association between them and afferent or efferent nerve fibres; though no nerve fibres can be identified in this embryo, there is an undoubted close connexion between the 4th neuromere and the acoustico-facial ganglion; connexion between the other cranial ganglia and the neuromeres is not so clear, there being one ganglionic mass related to more than one neuromere.

VASCULAR SYSTEM

Heart. As can be seen from the drawing of a model (Text-fig. 10), the heart consists of (1) a sinus venosus, in the upper left part of which is the opening into (2) the atrium which is, for the most part, a large single chamber, but which in its caudal part shows by an external groove and an internal ridge a commencing division into right and left chambers. The atrium leads by a constricted canal into (3) the ventricle, which forms the most caudal part of the heart and also shows the beginning of an interventricular septum and which then turns obliquely cranialwards and to the right; at the summit of its arch the ventricle joins (4) the bulbus, which passes caudalwards and to the left, extending from the most cranial limit of the heart to about half-way down it,

where it turns abruptly cranialwards into (5) the aorta. The sharpness of the various curvatures is emphasized more in the endothelial heart tube than in the myocardial. The caudal part of the sinus venosus lies in the septum transversum and is slung for some distance to the cranial side of its union with the



Text-fig. 10. Reconstruction of heart from in front. *A.* atrium; *A.c.* atrial canal; *V.* ventricle; *I.v.s.* interventricular septum; *B.* bulbus; *Ao.* aorta.

Text-fig. 11. Reconstruction of dorsal aortae, to show points of union, and the origin of ventral and lateral branches.

Text-fig. 11.

atrium to the middorsal line by a thick mesentery which is common to the heart and the gut, the heart lying ventral to the lung bud region, and the two organs being separated from each other only by the thickness of their respective walls. A mesentery slings the arterial end of the heart to the ventral body wall for a short distance until the aorta becomes embedded in the body wall. Elsewhere the heart tube is free.

With regard to structure; the sinus venosus is different from the rest of the heart and has not the same thick myocardial wall, but is more of the nature of a largely dilated vessel, it is indeed a true venous sinus.

In those parts of the heart where the walls are thin there is a considerable interval between the endocardial and myocardial heart tubes, which is filled with the loose trabecular tissue which has been so often described. In the thickest part of the heart, e.g. the ventricle, such a submyocardial space scarcely exists owing to the thickness of the muscular wall of the heart, and here the endocardial tube is formed of extremely thin, elongated cells placed end to end; where there is a large submyocardial space it can be seen that the trabecular tissue forms, on the deep surface of the space, a definite limiting membrane which abuts against the endocardial tube.

There is a difference in the appearance of the myocardium in the various parts of the heart; the sinus is by far the thinnest, and really has no myocardial coat at all; a myocardial coat can be recognized in the atrium, where it forms a fairly thick and compact layer of cells, quite different from what is found in the wall of the ventricle; in the ventricle the myocardium is thicker but the cells are more loosely arranged, they are large and form columns or chains enclosing clear spaces between them; the myocardium of the bulbus forms a layer intermediate in thickness between that of the sinus and the atrium.

Blood vessels. The general plan of the aorta can be seen in the figure (11). It passes cranialwards from the bulbus and then bifurcates, and the two divisions turn round in a caudal direction to form the first pair of aortic arches, and from the summit of each there arises the internal carotid artery. There is a second pair of aortic arches caudal to the first, and a third pair is almost completed by a considerable outgrowth from the ventral aorta and a much smaller ventral sprout from the dorsal aorta. The dorsal aortae then pass on along each side of the middle line as far as the caudal border of the 5th somite, or the junction of brain and spinal cord, where they join here and there; but true union does not occur till the level of the middle of the 7th somite, or at the level of the cranial end of the liver; from this point a single dorsal aorta passes towards the tail as far as the origin of the allantois, at the level of the 20th somite, where a division into two caudal aortae takes place, and here too the large umbilical plexus of vessels arises. The condition is thus rather in advance of that found in Girgis' (1926) embryo of 22 somites, and is very like that in Johnson's (1917) specimen of 24 somites.

Ventral branches, not strictly segmentally arranged, arise from the ventral surface of the aorta; there are 12 on the left side and 14 on the right. Dorsal and lateral branches are present also, but are not so definite as the ventral series. The picture formed by these various branches of the aorta and by the post-cardinal and sub-cardinal veins is almost identical with H. M. Evans' (1912) Fig. 436, p. 632, of the vessels in an embryo of 23 somites.

The more caudal of the ventral branches of the aorta, which rise in between the 20th and 23rd somites, i.e. between the 9th and 12th thoracic segments,

are those which give origin to the umbilical arteries; these two vessels run, one on each side of the allantois as it leaves the gut, but ventral and dorsal, the left being ventral, to the allantois as it lies in the body stalk; a branch connects the two arteries round the left side of the allantois just before its expanded end, and that is the only communication between the two vessels; the umbilical arteries then break up into branches in the chorion.

In the chorion there arise two veins which unite in the body stalk, like the limbs of a Y, to form a single large vessel, or blood lake, which occupies the concavity of the allantoic loop; this lake is then split into two, a right and a left umbilical vein, by the umbilical arteries which course through the blood lake on their way from aorta to chorion. The two umbilical veins then run towards the body of the embryo on each side of the allantois, which, together with the two arteries, separates the veins from each other. Beyond the body stalk the umbilical veins come to occupy the ventrolateral body wall, where they raise up large ridges which can be recognized in the intact embryo, the right vessel being the larger of the two; these ridges form the boundaries of the opening from the body cavity into the extra-embryonic coelom and have attached to them the line of amniotic reflection. The two veins then turn medialwards in the septum transversum, and are there joined by the vitelline veins, the combined vitello-umbilical trunk opening into the sinus venosus.

The anterior and posterior cardinal veins are not easy to follow; the anterior cardinal vein is formed by tributaries from the region of the optic vesicle and receives tributaries also from the dorsal region of the head; the vein then passes on the lateral side of the otocyst to the level of the 5th somite, where it turns ventralwards and is joined by the posterior cardinal vein to form the duct of Cuvier.

The posterior cardinal vein can be traced in a caudal direction as far as the level of the 17th somite, beyond which point it cannot be identified; it is placed dorsally between the Wolffian duct and the mesonephric vesicles.

The right duct of Cuvier opens independently into the sinus venosus on the cranial side of the vitello-umbilical trunk, whilst on the left side it joins the vitello-umbilical trunk before that vessel opens into the sinus. The vitelline veins enter the body of the embryo along the cranial wall of the yolk stalk and pass into the septum transversum. On careful examination of the septum, I was surprised to find how few are the vessels in it, there being scarcely any in the ventral part of the septum, but in its dorsal part there are two main vessels on the left side, whilst on the right side the arrangement is rather more complicated, the vitelline vein being broken up into several branches with which the liver cords are in contact, so that here and there a vessel is found to be surrounded completely with liver cords. But, as I have stated above, I do not believe that the liver cords have been the principal factors in the branching of the vitelline veins. The various branches of the vitelline veins ultimately unite into a single vessel on each side which joins the umbilical vein to form a vitello-umbilical trunk, and this, on the left side, is joined by the duct of Cuvier.

COELOM

The coelom consists of pericardial cavity and peritoneal cavity which communicate with each other through large pleuro-peritoneal canals and which are indirectly open to the extra-embryonic coelom round the attachment of the yolk stalk. The separation of the pericardial cavity from the pleuro-peritoneal canals is caused by a ridge on the lateral coelomic wall which overlies the duct of Cuvier, though it does not appear to be due to this vessel.

The two pleuro-peritoneal canals are four-sided tubes, open at the cranial end into the pericardial cavity through the dissolution of their ventral walls, and at the caudal end they open into the peritoneal cavity and the extra-embryonic coelom round the yolk stalk. Each canal has four walls, medial, lateral, ventral and dorsal; the medial wall is everywhere thicker than the others and is composed of about five layers of cells; the lateral wall is the thinnest; the ventral and the dorsal walls are rather thick and in the region of the septum transversum the ventral walls are raised into prominent ridges by the vitelline veins.

Cranially and caudally the ventral walls of the canals open out into the pericardial cavity and into the extra-embryonic coelom respectively, whilst more caudally the peritoneal cavity becomes closed off from the extra-embryonic coelom by the formation of the body stalk. The umbilical arteries, as they pass ventralwards from the aorta, give rise to ridges on the medial wall of the coelom and, as they run out into the body stalk, they cause similar ridges on the ventral wall. The caudal limit of the coelom is at the Wolffian ridges, to which reference has been made in connexion with the hind limb buds.

The septum transversum is an obliquely placed partition between the pericardial and peritoneal cavities, and it slopes from in front cranialwards and dorsalwards; growing into it from the dorsal side there is the liver; running along its caudal edge in a cranial and dorsal direction there are the vitelline veins, which form a complicated plexus in its substance; entering its lateral sides there are the umbilical veins caudally and the ducts of Cuvier cranially; the sinus venosus lies just above its cranial edge.

NOTOCHORD

The notochord begins, as an extension cranialwards of the primitive streak, as a thick, solid column of cells.

Its appearance in section varies at different levels; at the origin of the umbilical arteries it is an oval mass of cells, compressed from side to side and with an indication of a lumen in its interior; at the middle of the body stalk it has a circular outline and the lumen is quite definite; at the cranial edge of the body stalk the section is oval but compressed ventrodorsally, and at this level a distinct open space bounded by a limiting membrane separates the notochord from the marginal zone of the spinal cord, whilst between the notochord and the aorta there are a few scattered mesenchyme cells; at the middle of the

yolk stalk the notochord is flattened ventrodorsally, still has a lumen and is surrounded by the same space and limiting membrane, which are here in direct contact with the spinal cord dorsally and with the aorta ventrally; at the caudal part of the heart the notochord is rather smaller and more solid, with a less definite lumen, and mesenchyme cells again separate it from the aorta; in the lung region it is more circular in outline and is still further separated from the aorta; where it turns forward into the head region, and is cut along its long axis, it appears as two slender rows of cells with a cleft-like space between them; where the notochord is related to the brain it is circular in section till just before its end, where it is flattened from before backwards. It ends at the level of the pharyngeal membrane, being, as it were, squeezed out of existence by the brain wall coming into contact with the epithelium of the mouth in the infundibular region. The notochord comes into contact with the roof of the pharynx at two points; one, midway between the first and second pharyngeal pouches, and the other, midway between the second and third pouches; there is not any appreciable difference between the diameter of the notochord at these two points and elsewhere, nor does there appear to be any thickening of the underlying pharyngeal mucous membrane, as described by Davis (1923), and by Huber (1912) in a rather later stage.

GERM CELLS

An account of the appearance and arrangement of the germ cells in, among other specimens, an embryo of 4 mm., with 26–27 somites, has been given, and illustrated with beautiful photographs, by Politzer (1928*b*, 1933). He describes the germ cells as being situated both in the wall of the gut and in the adjacent mesoderm, from the caudal end of the intestinal portal to the origin of the allantois. Special cells in corresponding positions can be recognized in the present specimen, but they are not nearly so numerous, or, as is more probable, I have failed to recognize them, for there appear to be only about 40, whereas Politzer recognized 586, of which 330 where in the mesoderm and 256 in the entoderm. In the present embryo the majority of these cells are situated in the wall of the gut and these are recognizable more easily than those in the mesoderm; of those situated in the mesoderm, some occur alongside the gut in the splanchnic mesoderm, and others are occasionally found in the mesoderm just to the medial side of the mesonephric vesicles. They all are large cells with clear cytoplasm whereby, as Politzer describes, they may be differentiated from blood cells; the nucleus is large and round and has a granular appearance. A single germ cell is shown in Pl. I, fig. 6.

SUMMARY

An apparently normal embryo, nearly 3 mm. long, and with 25 somites, is described. It was obtained from a uterus removed at a post-mortem examination. It is hoped that the specimen may form a useful addition to a stage in human development in which there are not very many specimens available.

The following points of interest arise from a study of the specimen:

A convenient method of orienting the sections is described.

Photographs of the embryo, before and after the removal of the amnion, are shown.

The ovulation age has been estimated—by Streeter's method of the comparison of human developmental stages with those of *Macacus rhesus*—and the age of this specimen has been compared with that of other embryos of about the same stage of development.

The question of the interpretation of the morphological division of the somites is discussed.

The limb buds are in a very early stage of development, the forelimb bud being just recognizable, and it is suggested that there is no contribution from the myotome towards its structure.

The thyroid gland is smaller than usual, as far as its median component is concerned, whilst the lateral thyroid rudiment is well developed.

A very peculiar structure projects as a hollow tube into the interior of the pharynx; the author is unable to interpret it and has been unable to discover in the literature anything resembling it.

The lung buds are in an interesting stage and seem to throw some light on this region in other embryos of about this stage.

The allantois is exceptionally well developed, and there is a curious vesicle near its termination, which appears to have no connexion with the allantois.

The cloaca shows an early stage of division into urogenital sinus and rectum, by a pinching in of its lateral walls.

The urogenital system shows pronephric rudiments and mesonephric vesicles; the Wolffian duct is at a very interesting stage, not yet having reached the cloaca; it is suggested that the duct receives no contribution from the ectoderm.

The brain shows the neuromeres exceptionally well; ten of them are recognizable.

The heart forms an **S**-shaped loop; it is slung at its venous end by a dorsal mesentery.

The relation of the liver cords to the vitelline veins is discussed; it is suggested that the cords are not the main factor in the breaking up of the veins

Primitive germ cells are described and illustrated.

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EXPLANATION OF PLATE I

Fig. 1. Photograph of embryo, enclosed in amnion, in chorionic sac.
Fig. 2. Photograph of embryo, after removal of amnion.
Fig. 3. Photograph of a section through the middle of the vesicle, or "Epithelbläschen", near the end of the allantois in the body stalk. × 345.
Fig. 4. Photograph of part of section through a "cell island" of the chorion, with the syncytiotrophoblast intruding into the intervillous space; towards the right is a "giant cell". × 230.
Fig. 5. Photograph of part of section 152, to show a nephrostome; the section passes through the 3rd mesonephric tubule turning dorsalwards and lateralwards to join the Wolffian duct. × 580.
Fig. 6. Photograph of part of section 206, to show a germ cell in the wall of the gut on the left side. × 580.

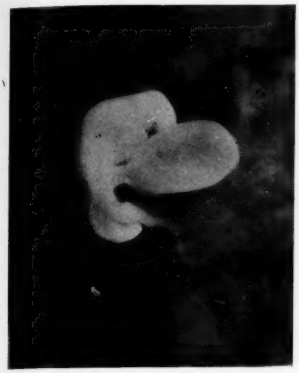


Fig. 1.

Fig. 2.

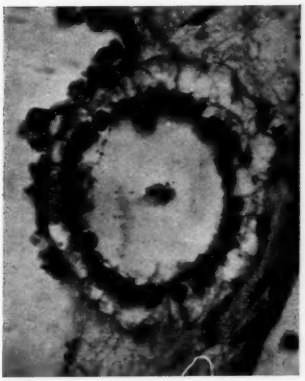
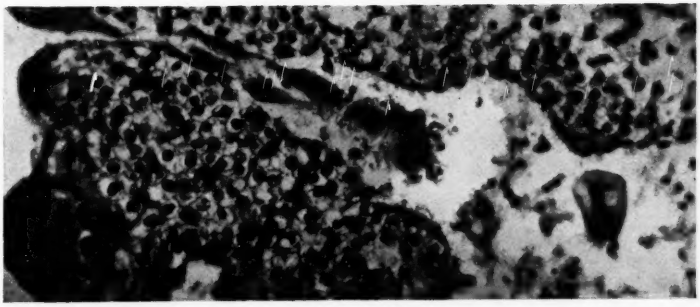


Fig. 3.



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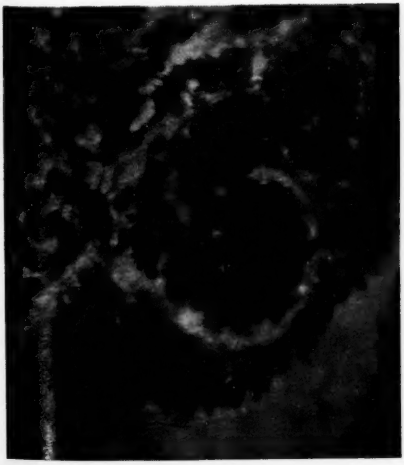

Fig. 5.

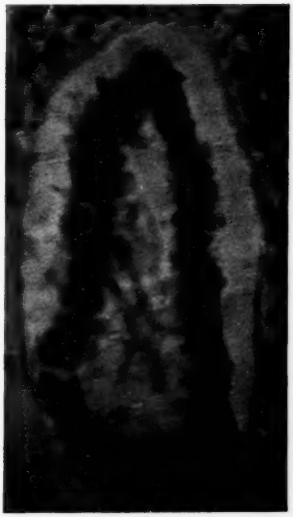

Fig. 6.

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THE DEVELOPMENT OF THE INFRA-UMBILICAL PORTION OF THE ABDOMINAL WALL, WITH REMARKS ON THE AETIOLOGY OF ECTOPIA VESICAE

By GEORGE M. WYBURN, M.B., Ch.B., F.R.F.P.S.G.
Senior Demonstrator of Anatomy in the University of Glasgow

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INTRODUCTION

The umbilical region of the human embryo has at all times attracted much interest as a prolific source of congenital defects and abnormalities, in spite of which, perhaps because of which, there still exists some gaps in our knowledge regarding the method of closure and completion of the belly wall.

An examination of the sections of a human embryo of 4·5 mm. in length (McIntyre II), with a favourable history, and showing the yolk sac with connecting duct, seemed to offer a fruitful field for study. The impossibility of limiting observation to the umbilicus alone was soon apparent, and it became obvious that satisfactory results could only be procured by a more comprehensive survey embracing the whole question of the development of the anterior abdominal wall.

The initial absence of a ventral wall to the embryo is found in all vertebrates with the exception of some of the Amphibia.

In the frog with its small, yolked, holoblastic egg gastrulation is complete—the blastopore forms at the equator with dorsal, lateral and ventral lips and wholly encloses the yolk cells of the vegetative pole. The yolk is thus taken into the body of the embryo and lies in the floor of the gut.

In the Elasmobranchs the large amount of yolk impedes cell division so that segmentation is meroblastic. The resulting blastoderm corresponds to the cells of the animal pole of the frog's egg. The embryo arises from the posteromedian portion of the blastoderm, so that only the dorsal lip of the blastopore is intra-embryonic, while the lateral and ventral lips, formed of the extra-embryonic

blastoderm, grow and enclose the yolk, the resultant sac being outside the body of the embryo to which it is connected by a stalk. Thus at an early stage the embryo is devoid of a ventral wall. The midgut, for a varying period, communicates with the yolk sac by the yolk stalk; this connexion is later obliterated.

In the Gymnophiona the anterior edge and the major portion of the lateral edges of the blastoderm take no part in the formation of the blastopore, and there is not only an absence of ventral wall, but the yolk remains uncovered.

The embryonic shield of the Amniota, according to Jenkinson, is the equivalent of the blastoderm of Gymnophiona. Blastopore formation affects only the shield, there being no true ventral lip. The edge of the extra-embryonic blastoderm grows steadily round the yolk entirely independent of blastopore formation, and finally encloses it at the vegetative pole. The yolk sac is extra-embryonic.

In the chick the enveloping blastoderm is continuous with the false amnion or chorion and is gradually separated from the yolk sac by the encroachment of the extra-embryonic coelom between somatopleure and splanchnopleure.

In placental mammals the egg has undergone loss of yolk and cleavage is accordingly holoblastic. The blastocyst with its surrounding trophoblast and enclosed embryonic material is possibly a modification to meet nutritional and protective requirements. The trophoblast, which from an early stage surrounds the yolk sac, is analogous with, if not the homologue of, the false amnion of the chick. This (the false amnion) is comparable to the extra-embryonic blastoderm of the Anamnia which encircles the yolk. The modified form of blastopore formation affects only the embryonic shield. The embryo is therefore without a ventral wall and the yolk sac is extra-embryonic. The completion and closure of the anterior abdominal wall occurs at a late stage of development in placental mammals.

It is the opinion of Keith that the trophoblast in the human blastocyst can be regarded as the precociously developed belly wall of the embryo. In view of the comparative lack of knowledge of the earliest stages in the formation of the human blastocyst, which acquires in a relatively short time its own salient characteristics, and taking into account the fundamental differences in the early ontogeny of the placental mammals which represent not a simple or common evolutionary plan but rather a great diversity of methods of attaining similar physiological requirements, it seems a hazardous experiment to affix a phylogenetic label to any portion of the human blastocyst.

In the heavily yolked eggs the inert yolk substance is responsible for the partial cleavage and limitation of gastrulation to the more active animal pole. The placental mammals show the early differentiation of the tissues concerned with protection and nourishment, and gastrulation is again much modified.

The absence of a true ventral wall in the early stages and the extra-embryonic position of the yolk sac may perhaps be regarded as a modification incurred by nutritional necessity, which modification could be explained as a

concomitant of incomplete and suppressed gastrulation following on the more prolonged embryological phase with its demand for "yolk" or "membranes".

The anterior abdominal wall in its development falls naturally into three parts—supra-umbilical, umbilical and infra-umbilical. The present portion of the work deals with the infra-umbilical abdominal wall.

THE INFRA-UMBILICAL ABDOMINAL WALL

This part of the ventral wall cannot be said to exist until the appearance of the genital tubercle with the subsequent development of an interval between the tubercle and lower attachment of the body stalk. In the series examined the interval first becomes apparent in an embryo of 12·5 mm. which shows a well-marked genital tubercle with a narrow dense band of mesoderm between it and the attachment of the stalk. Keibel & Mall give the first appearance of the tubercle as occurring in an embryo of 13 mm. This mesoderm, caudally merging into the genital tubercle and superiorly continuous with the tissue of the stalk, would therefore seem to be the basis of the future infra-umbilical portion of the belly wall.

An endeavour to trace the origin of this mesodermal mass through a series of younger embryos in the Glasgow Collection has involved a consideration of the primitive streak, of the cloacal membrane, of the mesoderm in general, and has led to a study of existing descriptions of early embryos in connexion with the individual points within this question.

EMBRYO McINTYRE I

The first of the series examined was embryo McIntyre I. Observations on this embryo were published by Bryce (1924), but in his paper particular interest was centred on the more cranial sections of the embryonic shield. The embryo is of the presomite class, having a pronounced ventral curve at the hinder end, a primitive streak measuring 0·33 mm., the major portion of which occupies the ventral aspect of the caudal flexure, and a total length of 1·4 mm., including the primitive streak.

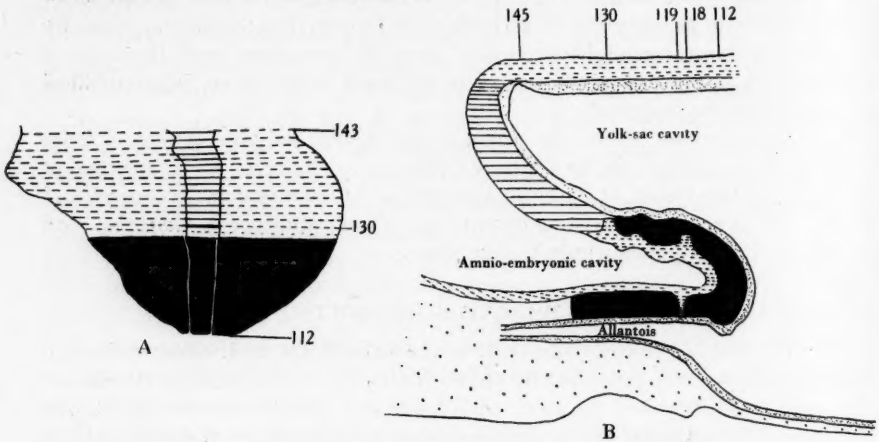
Bryce is of the opinion that the McIntyre embryo is younger than embryo Gle (Graf v. Spee) and embryo Vull (Éternod), to which it bears a close resemblance and is probably a stage succeeding that of Ingalls in which, however, the disc has the dimensions of $2 \times 0{\cdot}75$ mm.

A graphic reconstruction was made of the caudal half of the embryo from sections 109–150 (Text-fig. 1A, B), the exact position of the sections being indicated on the drawing of the wax models (Pl. I, fig. 1).

Section 146 (Pl I, fig. 3) is placed at the flexure and shows the opening of the archenteric canal. Sections 150–147 are through the region of the caudal bend, consequently there is some difficulty in interpreting the nature of the tissues and it is impossible to discriminate between ectoderm and mesoderm, but from the position it is probable that the section is cut through Hensen's knot.

Cranial to 146 the sections show the primitive groove and primitive streak on the ventral aspect, i.e. on the caudal portion of the shield which has been bent round ventrally to become continuous with the body stalk.

From sections 145 to 142 (Pl. I, fig. 4) the primitive streak appears as a fusion between the ectoderm and mesoderm. The endoderm dorsally is in contiguity with the mesoderm, but there is no distinct fusion of the layers. In the sections cranial to 142 the primitive streak on the ventral aspect has the appearance of mesoderm budding off from the ectoderm, more especially from the walls of the primitive groove. The endoderm forms a distinct layer connected to the mesoderm by a few cellular strands. This appearance persists in the next twelve sections, the only change being a gradual deepening of the yolk-sac pocket with consequent approximation of endoderm and ectoderm, and decrease of intervening mesoderm.



Text-fig. 1. Embryo McIntyre I. ×100. The numbers refer to the sections. A. Reconstruction of the surface of the caudal portion of the embryonic shield, i.e. the portion bent ventrally to form the tail fold. In the midline is the primitive groove. Area in which mesoderm crosses the midline is shown in black. B. Reconstruction of a median sagittal section (see Pl. I, fig. 1). Description in text. Ectoderm—interrupted horizontal lines; endoderm—fine stippling; dense mesoderm—black; primitive streak—horizontal lines.

Section 130 (Pl. II, fig. 5). The primitive groove is still present. The ectoderm forms a thickened cord of cells with the endoderm lying immediately above. There is no intervening mesoderm, but mesoderm is seen extending laterally on each side, separated by an interval from the ectoderm and endoderm. The appearance is that of a cloacal membrane, and extends only over one section.

Sections 129–127 (Pl. II, fig. 6) again show mesoderm between the ectoderm and the endoderm with a gradual flattening out of the primitive groove. The amount of mesoderm between the two layers gradually increases. There is

no sign of budding off of the mesoderm from the ectoderm, and while the three layers are in contiguity at the midline this may be in some measure artificial, as both ectoderm and endoderm can be traced as distinct layers while the mesoderm stretches across from side to side between them.

Section 126. There is no primitive groove. The ectoderm seems to be thickened in the midline, forming a plate of cells which at first glance would appear to extend up to and establish contact with the endoderm. On further examination with an apochromatic 4 mm. lens this resemblance to a cloacal plate is found to be due to shrinkage of the mesoderm on each side leaving an apparent space between the "plate" and the mesoderm. Similar spaces can be seen in the mesoderm more laterally as a result of formalin fixation. The ectoderm and endoderm can again be traced as distinct layers across the middle line.

Section 125 (Pl. II, fig. 7). Is similar to 126, but the resemblance to a cloacal plate is even more striking.

Sections 124–120 (Pl. II, fig. 8). Mesoderm is again visible separating the ectoderm and endoderm.

Section 119 (Pl. III, fig. 9). The amniotic space between the disc and body stalk is diamond-shaped in the section. From the lower apex a distinct cord composed of a double row of cells can be made out extending down to and establishing contact with the endoderm of the allantois which at this level is oval in section and extending up the cord.

Section 118 (Pl. III, fig. 10). The amniotic space is still diamond-shaped. The cord of cells noted in the previous section is not now evident, the amnion and allantois being separated by definite mesoderm. From the upper angle of the space a short process extends up to but does not reach the endoderm of the yolk-sac diverticulum, a single row of mesodermal cells intervening.

Sections 117–109 (Pl. III, fig. 11). The amniotic space gradually diminishes and disappears, the mesoderm of the disc fusing with that of the body stalk, the resultant mass being placed between the endoderm of the yolk sac dorsally and that of allantois ventrally.

Bryce (1924) in the description of the embryo states that the primitive streak extends up to section 117, from which point it is replaced by the cloacal plate connecting the yolk-sac diverticulum with the ectoderm and found up to the commencement of the allantoic duct proper. As before mentioned, in the original description, interest was focused almost entirely on the cranial sections and not on the region now examined.

No evidence of a cloacal plate is to be found in section 117, or those cranial to that; in fact in those sections where the amniotic space is gradually closing in, the ectoderm can be distinguished only with difficulty and ultimately disappears entirely.

Definite contact between ectoderm and endoderm is established at two points, viz. sections 130 and 119. The possibility of a cloacal plate in sections 125 and 126 has been rejected because of the alternative explanation of an artefact during fixation. In this embryo there are therefore two points at which

an undoubted cloacal membrane can be said to exist. Between these two points mesoderm is continuous on the one hand with that of the primitive streak and on the other with the mesodermal basis of the body stalk.

Regarding these findings there are two questions which occur—(A) the source of the mesoderm of the body stalk; (B) an explanation of the division of the cloacal membrane.

SOURCE OF MESODERM OF THE BODY STALK

It is firstly necessary that there should be a clear conception of the structure of the primitive streak.

Bryce (1924) states that all three layers are fused over the length of the streak, but while the mesoderm can be observed budding off from the ectoderm, the basal part of which is indistinguishable from that layer, the endoderm, except in the most caudal section where it is not present, preserves its identity and can be traced as a distinct layer across the middle line. There is certainly no interval between the mesoderm and endoderm, but in none of the sections of the primitive streak did there appear to be any actual budding off of mesoderm from endoderm.

Hill & Florian (1931), describing the primitive streak of the "Dobbin" embryo (0·98 mm.), state that the endoderm is clearly visible on the ventral aspect of the streak. Ingalls (1918) in his embryo of 2 mm. describes a considerable interval between the endoderm and the primitive streak, while Heuser (1930), in one of 1·6 mm., shows the streak as a fusion of ectoderm and mesoderm, the endoderm lying quite free. Debeyre (1912), on the other hand, states of the region of the primitive streak: "Tout le long de la ligne primitive ou à son niveau on remarkera l'union intime qui existe entre les feuillets." Florian (1934), from reconstructions of embryos WO, Op. and Fetzer, is of the opinion that in the primitive streak primordium there is a fusion of endoderm and mesoderm in the midline and that this persists for some time, while Rossenbeck (1923), writing on the embryo Peh-Hochstetter, considers the primitive streak as a fusion of ectoderm and mesoderm, the endoderm being separated by an interval from the actual primitive streak.

This disparity in the description of the character of the streak is probably due to variations in its form according to the degree of development, not necessarily corresponding to the size of the embryonic shield; but most observers seem agreed that while there is a fusion of all three layers in the region of Henson's knot, the actual streak itself is represented by a fusion of ectoderm and mesoderm. Grosser (1927) expresses the opinion that the primitive groove is due to a sinking of the ectoderm on account of the heavy emigration of mesodermal cells.

The blastocyst of man and higher mammals is characterized by the precocious development of mesoderm, which being in the later stages an angio-

blastic tissue arises in accordance with the mode of nutrition of the embryo (Bryce).

Of three of the earliest ova showing the embryonic rudiment, in that of Peters (1899) the amniotic and yolk-sac vesicles are separated by mesoderm except in the middle line at the cranial end; in T.B. 1 (1908) there is a considerable interval between the two vesicles which are situated in a cavity already filled with mesenchyme—the unusual distance between the amniotic cavity and the yolk sac may be to some degree artificial; Streeter (1927) in a description of section 4 of the Miller ovum—the sixth section through the rudiment—states that the amniotic and yolk-sac cavities are in a common compartment surrounded by mesenchyme, the ectoderm of the amnion being in contact with the endoderm of the yolk sac with but a narrow intervening cleft. There is, however, some doubt as to what constitutes the yolk sac in the Miller ovum.

Fetzer (1910), describing an embryo of 0·23 mm. in length, states that there is no primitive groove, medullary fold, or neurenteric canal. Nevertheless the yolk-sac endoderm and ectoderm of the shield are separated by a layer of mesenchymal cells passing towards the cranial end and fusing in the midline with the ectoderm. According to Rossenbeck this is undoubtedly the primitive streak.

Graf von Spee, discussing Peter's ovum, is of the opinion that the anterior and posterior poles of the amniotic and yolk-sac vesicles grow into the solid mesenchyme separating them from the chorion which mesenchyme, acting as a wedge, ultimately completely separates the ectoderm and endoderm except for an area in the midline at the caudal end—the cloacal membrane.

It is now well known that the primary mesenchyme in the human ovum and that of the higher primates makes its appearance long before there is any indication of a primitive streak, thereby differing fundamentally from that in the lower mammals.

Streeter (1927) is of the opinion that this mesenchyme arises either from the inner cell mass during the formation of the segmentation cavity or alternately is split off from the trophoblast. The close relation of the mesenchyme to the trophoblast inclines him to the latter explanation.

Bryce (1924) accepts the former hypothesis and regards the differentiation of the primitive mesenchyme as simultaneous with that of ectoderm and endoderm from the formative cell mass.

Strahl & Beneke (1910) express the opinion that the primary mesoderm is split off at the morula stage, the ectoderm and endoderm remaining widely connected in the early stages. Later this connexion is restricted to the primitive streak region.

Hubrecht (1909) finds that in *Tarsius* the mesoderm of the connecting stalk is derived from a proliferation of the ectoderm at the posterior margin of the shield which Bryce (Quain) suggests is a precocious development of the primitive streak formed later as an extension of this area.

In a history of the primates Hill (1932) shows four stages in the genesis of the body stalk:

(1) Lemur, e.g. *Loris*, where the mesoderm arises relatively late (blastocyst 3 mm.) and both extra- and intra-embryonic mesoderm are derived from a primitive streak. The mesenchyme of the allantoic outgrowth which is the homologue of the connecting stalk is formed from the primitive streak, arising from its hind end and extending round the cloacal membrane.

(2) In *Tarsius* the connecting stalk mesenchyme is in the first instance a proliferation of the posterior margin of the shield ectoderm, later being reinforced by a backward extension from the primitive streak.

(3) In "*Hapale*" embryo there is an area of proliferating mesenchyme at the caudal margin of the shield which Hill suggests corresponds to the precociously developed hinder end of the primitive streak in *Tarsius* and which in his opinion forms the whole of the primary mesoderm, including that of the stalk. The extra-embryonic mesenchyme is differentiated some considerable time before the appearance of the primitive streak.

(4) The fourth stage is the human embryo.

Florian (1930) has demonstrated the existence of a proliferating area in the posteromedial margin of the shield ectoderm in the embryo Fetzer and believes this to be the source of the stalk mesenchyme and the major portion of the rest of the primary mesenchyme in the human. In a recent publication (1933) he avers to have satisfied himself as to the existence of such a region in the embryos Peters, Werner, and von Mollendorff's Wo and Op. This accords with the views of Rossenbeck that in the human the primitive streak forms relatively little of the mesoderm.

It is to be noted that in the embryos of *Loris*, *Tarsius* and Selenka's Kleim S., the ectoderm and endoderm of the rudiment are in contact in the early stages; the duration of contact diminishes as the scale is ascended.

In answer to question A (p. 206) it can therefore be stated that in the human the mesenchyme of the stalk is in all probability derived from a proliferation of the caudal margin of the shield ectoderm, and one can postulate a stage, perhaps transient, when the ectoderm and endoderm are connected in the midline over a considerable extent of the embryonic shield. As seen in the sections of McIntyre I and also demonstrated by Florian (1934) in embryo Beneke (size 0·75 mm.) and by Hill and Florian (1931) in the Dobbin embryo (0·98 mm.), the mesoderm of the primitive streak is continuous round the cloacal membrane with that of the stalk.

As there is no histological difference between the primary as compared with the secondary mesoderm it is impossible from a study of the sections to say where the one ends and the other begins, but it is reasonable to assume that the mass of mesoderm beyond the cloacal membrane and on the caudal aspect of the stalk is compounded of primary and secondary mesoderm.

DIVISION OF THE CLOACAL MEMBRANE

Until the recent work of Florian, scant attention was given by embryologists to the caudal region of the human embryo. Any semblance of union between the ectoderm and endoderm in the expected region by direct apposition or indefinite cords of cells was designated "cloacal membrane". The approximation of endoderm and ectoderm with no intervening mesoderm in section 130 of McIntyre I gives the appearance of an undoubted cloacal membrane. Similarly the connecting cord of cells from the amniotic ectoderm to the endoderm of the allantois is clearly demonstrable in section 119. On the other hand, a cursory examination of sections 125 and 126 might lead to their interpretation as cloacal plates, whereas a more careful study shows the impossibility of excluding mesoderm intervention.

Section 118 with the cord of cells from the amnion extending almost to the endoderm suggests that at this point former fusion between the two layers is being broken down by a pressing in of mesoderm from each side.

Most observers are agreed as to the difficulty in defining the limits of the cloacal membrane. Hill & Florian (1931), describing the Dobbin embryo, comment on this, more especially the differentiation between the membrane and the primitive streak. Of this region Debeyre (1912) says: "A son extrémité postérieure comme dans l'embryon de Keibel-Frassi (1907) se trouve l'ébauche d'une membrane cloacale très probablement au moins (bien que nous ne puissons le vérifier directement sur les coupes)." Much of this difficulty, however, arose from the failure to recognize the incompleteness of fusion in many parts of the "membrane".

Sternberg (1927), in an embryo of 4 somites, described a fusion of ectoderm and hindgut endoderm, and also separate connexions between amniotic ectoderm and the endoderm of the allantois. Since then similar conditions have been noted in other specimens—Florian (1930) in embryo Bi II (also of 4 somites); Hill and Florian (1931) in the Dobbin embryo; and West (1930), describing an embryo of 8 somites, states that the cloacal membrane is very indefinite, actual contact being established in only two sections.

In the Ingalls (1918) embryo there exists what the author designates as an amnio-allantoic duct characterized by a pocketing of the amniotic cavity towards the allantois with contiguity of ectoderm and endoderm. Rossenbeck (1923), after noting the cloacal membrane as a cord of cells from the ectoderm to the endoderm in the Peh-Hochstetter embryo, in a subsequent paragraph describes an apposition of the dorsal wall of the allantoic passage with the floor of the amniotic cavity.

From a study of McIntyre I, along with the facts available from recent work on this subject, more especially the researches of Florian, I am of the opinion that the primordium of the cloacal membrane is in the early stages relatively extensive, the caudal fusion of endoderm and ectoderm being between the amnion and the future allantois, at this stage not yet separated off from the

Table I. *Table showing extent of cloacal membrane relative to the size of the embryo*

Embryo	Length of embryonic disc	Cloacal membrane
	Presomite	
W.O. (V. Mollendorff, 1925)	0·25 mm.	What von Mollendorff describes as the cloacal membrane is, according to Florian, the primitive streak, and von Mollendorff's allantois is the cloacal membrane of Florian
"Fetzer" (Fetzer & Florian, 1930)	0·26 mm.	The cloacal membrane is separated by an interval from the primitive streak
T. F. (Florian (1928))	0·4 mm.	The cloacal membrane is a cord of cells which extends on to and connects the allantoic endoderm with the amniotic ectoderm
Bi I	?	Primitive streak reaches the cloacal membrane
Bi 24	0·625 mm.	Length 0·06 mm.
"Hugo" Stieve (1926)	0·635 mm.	Length 0·035 mm.
"Beneke" (Strahl & Beneke, 1910)	0·75 mm.	Cloacal membrane separated by an interval from the primitive streak
W.A. 17 (Grosser, 1930–1)	0·85 mm.	Length 0·13 mm. and extends on to the allantois
"Dobbin" (Hill & Florian, 1931)	0·98 mm.	Length 0·08 mm.
Peh-Hochstetter Rossenbeck (1923)	1·4 mm.	Cloacal membrane extends well on to allantois
Heuser (1930)	1·6 mm.	Cloacal membrane extends well on to allantois
Ingalls (1918)	2·0 mm.	Length 0·12 mm., and there is an additional connexion between the allantoic endoderm and amniotic ectoderm
	Somites	
F. von Orts Llorca (1934)	2	Cloacal membrane in two parts—one of which is allantoic
H 3 (Wilson)	2–3	Cloacal membrane at the commencement of allantois
Bi II	4	Cloacal membrane broken up—one portion involving allantois
Sternberg (1927)	4	Cloacal membrane is in four portions
Pfannenstiel-Krömer	5–6	Very short cloacal membrane extending some distance into the allantois
Dandy (1910)	7	Not mentioned
Payne (1925)	7	Not mentioned
West (1930)	8	Length 0·09 mm.
Eternod (1899)	8	Length 0·05 mm.
Corner (1929)	10	Cloacal membrane terminates before reaching the allantois
Embryo Bl. XI	10	Cloacal membrane terminates before reaching the allantois
Pfannenstiel III	13–14	Has trebled its length compared to Pfannenstiel-Krömer
Heuser (1930)	14	Length 0·18 mm.
Atwell (1930)	17	Length 0·18 mm. Extends to the allantois
Davis (1924)	20	Length 0·16 mm.

yolk sac. At a subsequent period the allantoic portion of the connexion is broken down by an extension of the mesoderm in towards the middle line, the chordal[1] part remaining as the site of the permanent cloacal membrane. This undergoes extension later along with the growth of the cloaca and embryo as a whole.

Table I is a series of embryos arranged according to size, with, as far as can be obtained, the available data regarding the cloacal membrane.

In the earliest specimens before the appearance of the allantois the fusion of ectoderm and endoderm occurs at the caudal end of the disc cranial to the attachment of the body stalk. In later embryos of the presomite class, in every case the membrane includes the allantois, and this condition remains up to the stage of about 4 somites. At this time the connexion with the amnion is being interrupted by mesoderm, and in the immediately older embryos of about 5–8 somites the cloacal membrane will be relatively much reduced. This is exemplified in the Pfannenstiel-Krömer embryo of 5–6 somites, and may account for its apparent absence in that of Dandy (1910)—7 somites—and Payne (1925)—7 somites. After the 10-somite stage the cloacal membrane seems to undergo proportionate development, although according to Heuser there are variations in its extent which have no relation to the size of the embryo.

The inferences deduced from the table, owing to the small series and lack of mathematical measurements, can only of course be accepted in a broad sense, but are of interest as coinciding with the suggestions made from observations on embryo McIntyre I. It should be noted that in the Dobbin embryo Hill and Florian observed the presence of granules in the cells of the cloacal membrane which in the opinion of Florian are to be regarded as indicative of degeneration of the cells. These granules occurred in the areas of incomplete fusion. F. von Orts Llorca (1934) has demonstrated a very similar condition to that in in McIntyre I in an embryo of 2 somites where he shows the cloacal membrane in two parts, (1) at the junction of allantois and yolk sac, (2) between the amnion and allantois. Regarding the membrane he states: "Da in dieser Gegend zahlreiche absterbende Zellen vorhanden sind, scheint es, dass die Kloakenmembran in frühen Entwicklungsstadien eine grössere Ausdehnung besitzt und dass sie zunächst bis auf das Allantoisdivertikel übergreift, während später der der Allantois entsprechende Teil der Kloakenmembran verschwindet und das Mesoderm zwischen das Amnion- und das Allantoisepithel vordringt."

The terminal point of the primitive streak is still a matter of controversy. In embryo McIntyre I, as already noted, sections 142–131 show the mesoderm distinctly budding off from the ectoderm of the primitive groove, whereas in the sections after the first part of the cloacal membrane, i.e. in 129–119, while the mesoderm and ectoderm are in continuity there is no actual formation of

[1] In this paper the portion of the cloacal membrane nearest the body stalk will be designated the "allantoic end", and the portion most distant from the body stalk the "chordal end".

mesoderm. One would therefore infer that the primitive streak terminates at the cloacal membrane.

Keibel and Elze express the opinion that the cloacal membrane should be regarded as a modified part of the primitive streak which reaches up to the body stalk. Their views are based on the presence of thickening of the ectoderm which stretches down almost to the allantois. This I would regard as a partially obliterated cloacal membrane and not primitive streak. Grosser (1927) implies that the streak becomes divided, a part growing into the tail bud, and the remainder being represented by the cloacal membrane. He also states that the primitive streak does not appear until the disc is 0·5 mm. long. The cloacal membrane has been shown to exist when the anlage of the primitive streak is represented only by a nodal fusion of all three layers (Florian, 1934). In the same monograph Florian has also demonstrated in E. Beneke and Fetzer an interval between what he regards as the cloacal membrane and the primitive streak in which all three layers are distinct and separate. In the later stages the primitive streak grows down to the cloacal membrane.

The name primitive streak was first given to the appearance observed in the chick embryo, the "streak" being essentially caused by the formation of mesoderm from the ectoderm at that particular region. As there is no mesoderm in the formation of the cloacal membrane it can be assumed that the actual streak as observed in the chick does not include the membrane. Keibel argues that the membrane is a secondary formation, the approximation of the ventral rim of the cloaca and ectoderm pressing the mesoderm from the midline. Recent work has negatived this as the union of ectoderm and endoderm at the caudal end of the shield occurs as early as, if not before, the primordium of the primitive streak.

Grosser (1927) inclines to the view that the ectoderm and endoderm of the embryonic disc are everywhere separated by an interrupted layer of primitive mesoderm, but quotes von Mollendorff's assertion of a ribbon-like connexion between the layers in the midline caudally which Rossenbeck regards as a connexion from the morula stage.

Strahl & Beneke (1910) express the opinion that the ectoderm and endoderm remain widely connected in the early stages, this connexion being later restricted to the primitive streak region.

Opinion seems unanimous that some degree of contact is maintained in the midline, and it is not unreasonable to conclude that in man as in lower mammals there is continuity along the length of the disc, separation being effected as suggested.

The hypothesis advanced here is as follows. Despite the precocious development of the primary mesenchyme in the human, it seems reasonable from phylogeny to postulate a stage when the ectoderm and endoderm are in contact in the midline. The appearance of the primordium of the primitive streak representing the fused lips of the blastopore acclaims the formation of secondary mesoderm separating the ectoderm and endoderm in this region.

The invagination of the dorsal lip with the pushing forward of the head process intervenes between the two layers of the disc cranially. Anterior to the head process the layers remain in contact—the site of the buccopharyngeal membrane.

Caudal to the fused lips of the blastopore and reaching to the attachment of the stalk remains a relatively extensive area of contact, the anlage of the cloacal membrane. The yolk-sac endoderm of the caudal portion of the membrane is predestined to become the epithelium of the allantoic diverticulum, and, as suggested above, the ectoderm and endoderm are here later separated by incursions of mesoderm. The cloacal membrane would thus be the homologue of the ventral lip of the blastopore and adjacent area.

From the cranial end of the primitive streak wings of mesoderm extend forwards on each side of the head process fusing anteriorly in the region of the mesodermal field of the protocardial area. Similarly from the caudal end of the streak as seen in McIntyre I, mesodermal processes extend round the cloacal membrane fusing with the stalk tissue in the region of the posterior mesodermal field. There would therefore be a close analogy in the development of the poles of the disc, the more rapid progress of the caudal end in the human obscuring many of the features.

The above assumption is compatible with the facts as observed at a later stage of development.

The bearing of these findings and conclusions on the problem of ectopia vesicae will be discussed later.

DESCRIPTION OF EMBRYOS FROM 2·4 TO 40 MM.

2·4-*mm. embryo*

The next of the Glasgow series to be examined is an embryo of 2·4 mm. in length. This was obtained as a result of abortion, and although seeming for the most part morphologically normal the tissues show some pathological change and the region of the tail fold is abnormal. This and the plane in which the sections were cut forbid any definite statement regarding the position and size of the cloacal membrane.

A photograph of a wax plate model of the embryo is given in Pl. III, fig. 12, and it is evident that there is some degree of torsion on a longitudinal axis, the attachment of the stalk and yolk sac being twisted to the right, while the caudal extremity turns to the left.

Section 84 (Pl. I, fig. 2) shows a mesodermal mass on the caudal aspect of the stalk attachment, and the graphic reconstruction of a coronal and sagittal plane give some idea of the extent of this tissue (Text-fig. 2A, B).

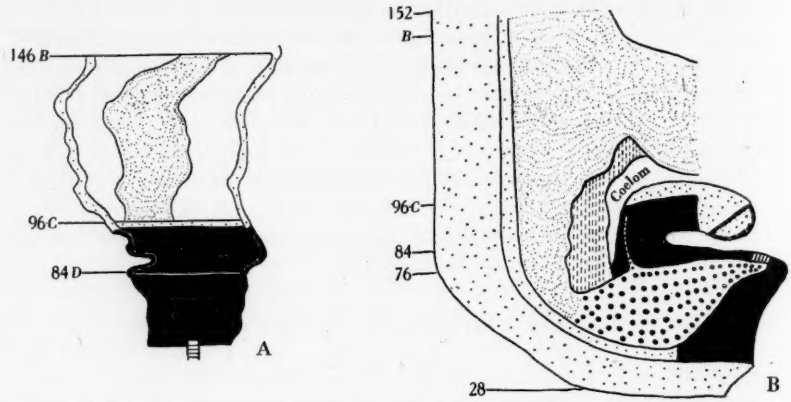
It envelops the embryonal end of the stalk, extending for a short distance only into the stalk substance and becoming continuous below with the mesoderm forming the ventral wall of the caudal end. As has been stated, the mesodermal mass is composed of primitive and secondary mesoderm (the secondary

mesoderm extending in wing-like processes round the cloacal membrane), the two fusing and forming the tissue seen in the figure, and illustrated in the reconstruction.

It is to be noted that at this stage there is no anterior abdominal wall, the entire ventral aspect below the septum transversum being occupied by the wide communication with the yolk sac and the attachment of the stalk. The allantoic end of the cloacal membrane is some distance from the stalk attachment.

4·5-*mm. embryo* (*McIntyre II*)

This embryo was obtained following a hysterectomy and sterilization in a case of mitral stenosis and incompetence with a history of cardiac trouble during previous pregnancies. When brought into the Department the vesicle



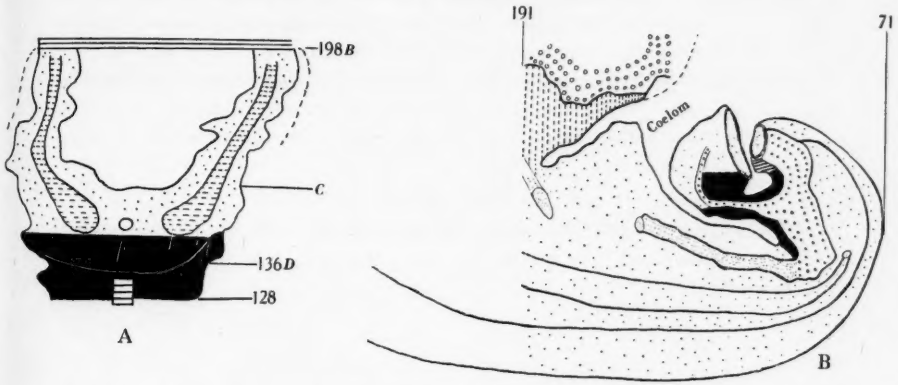
Text-fig. 2. Embryo 2·4 mm. ×50. Numbers refer to the sections. A. Reconstruction of the ventral surface from the septum transversum to the cloacal membrane. B. Reconstruction of a median sagittal section (see Pl. III, fig. 12). Description in text. Gut and yolk sac—fine stippling; cloaca and allantois—large dots; cloacal membrane—straight lines; dense mesoderm—black; splanchnopleure—interrupted lines. Between *B* and *C* the ventral wall is occupied by the mouth of the yolk sac. *D.*=the level of the caudal attachment of the body stalk.

was perfectly clear and transparent with no magma present. On opening the vesicle the umbilical arteries could be seen actually pulsating. There is therefore every probability of a normal structure, and considerable weight may be attached to the findings in this case.

Pl. VI, fig. 21, is a photograph of a wax-plate reconstruction from which it is observed that the ventral wall is still occupied by the communication with the yolk sac considerably narrowed from that in the 2·4 mm. Below this is the stalk attachment.

The embryo was cut transversely in a cranio-caudal direction, and Text-fig. 3A shows a graphic reconstruction of the ventral surface from the septum

transversum to the region of the cloacal membrane. The caudal end is represented straightened out and the approximate position of the cloacal membrane indicated. Text-fig. 3B is a graphic reconstruction in a plane indicated in Pl. VI, fig. 21. The cloacal membrane extends over twenty sections, each of 10 μ, and has therefore a total length of 0·2 mm. Owing to the direction of the sections and the folding of the caudal end, the distance of the membrane from the stalk attachment cannot be accurately measured, but it is apparent from a study of the reconstruction that the allantoic end of the cloacal membrane is some distance below the lower attachment of the stalk.



Text-fig. 3. Embryo 4·5 mm. (McIntyre II). ×50. The numbers refer to the sections. A. Reconstruction of the ventral surface from septum transversum to cloacal membrane. The tail is represented straightened out. B. Reconstruction of median sagittal section (see Pl. VI, fig. 21). The yolk sac is not shown. Description in text. Gut—fine stippling; cloaca and allantois—large dots; dense mesoderm—black; cloacal membrane—horizontal lines; allantoic vessels—interrupted horizontal lines; septum transversum—interrupted vertical lines; liver—small circles. The interval between *B* and *C* is the communication between extra- and intra-embryonic coelom. *D*=the level of the caudal attachment of the body stalk.

Section 136 (Pl. IV, fig. 13) shows the lower attachment of the stalk and the hindgut with its dorsal mesentery. There is no indication of the cloacal membrane, indeed the cloaca itself only comes into view in the more caudal sections.

Pl. IV, fig. 14, is a photograph of section 131 and is the allantoic end of the cloacal membrane. It can be seen that the section is at the head of the tail fold and some distance from the stalk attachment.

Section 138 (Pl. IV, fig. 15) is at the caudal limit of the attachment of the stalk. There is a strong mesodermal condensation at the embryonal end of the stalk. Section 144 (Pl. IV, fig. 16) is the site of the cranial attachment, and the loose texture of the tissue is apparent in comparison with that in section 138.

This mesodermal condensation at the lower aspect of the stalk, as can be seen from the reconstructions, is continuous with and prolonged into the tissue of the ventral wall below the stalk attachment, extending down to the cloacal

membrane. It has increased relatively from the corresponding mass in the 2·4-mm. embryo and represents the primordium of the genital tubercle and ventral parts of the infra-umbilical abdominal wall.

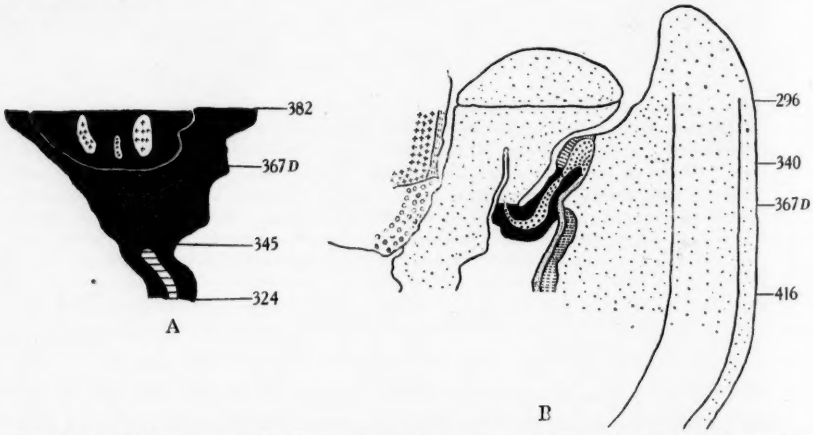
Keibel & Mall give a reconstruction of the embryo Pfannensteil-Kromer of 1·38 mm., showing a cloacal membrane of 0·02 mm. length extending up to the attachment of the stalk, and also a reconstruction of Pfannenstiel III of 2·6 mm.—with a cloacal membrane 0·13 mm. long extending up to and a short distance along the body stalk. Most authors seem to be in agreement with this, including Bryce (Quain's *Anatomy*, vol. I), Keith and Frazer.

In the embryo just examined there is quite a definite interval between the cloacal membrane and the stalk. In one of his later publications a similar mesodermal separation was shown by Keibel in embryo "EB", and has been demonstrated by Sternberg (1926) in embryo B of 18 somites and embryo F of 4 mm.

The question will be later discussed in relation to the problem of extroversion of the bladder.

7-mm. embryo

This specimen was obtained following curettage and cut transversely at 10 μ in a cranio-caudal direction. Pl. V, fig. 17, is a drawing of a wax model reconstruction.



Text-fig. 4. Embryo 7 mm. Numbers refer to the sections. A. Reconstruction of the caudal portion of the ventral surface. × *circa* 50. The tail is straightened out. B. Reconstruction of a median sagittal section. × 25. See Pl. V, fig. 17. Gut—fine stippling; cloaca and allantois—large dots; dense mesoderm—black; cloacal membrane—horizontal lines; mesentery—interrupted horizontal lines; septum transversum—interrupted vertical lines; liver—small circles; heart and allantoic vessels—crosses. *D* = the level of the caudal attachment of body stalk.

The cloacal membrane stretches over sixteen sections, giving a total length of 0·16 mm. Section 329 illustrates the chordal end of the membrane, and section 345 the allantoic end (Pl. V, figs. 18–20), and it is to be noted that in

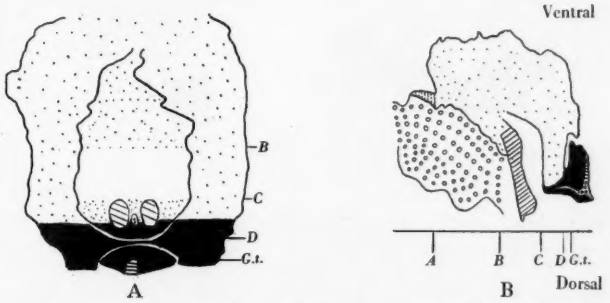
this specimen there is no membrane as represented by the coaptation of two layers of cells, but rather as a cord or plate of cells several rows thick, the major portion of the plate seeming to be derived from the endoderm. Section 367 is through the caudal attachment of the stalk; the allantoic end of the cloacal membrane is therefore 0·24 mm. from the stalk.

Section 373 (Pl. V, fig. 19) affords comparison between the caudal and cranial regions of the body stalk, the former exhibiting a fairly dense mesenchyme around and on the superficial aspect of the vessels, while the latter shows the typical loose texture of stalk tissue.

As can be observed in the graphic reconstruction of a medial sagittal section (Text-fig. 4A, B), the mesodermal condensation in the stalk is continuous with and merges into the dense tissue intervening between the ventral part of the cloaca and the surface. It extends on either side of the cloacal membrane as part of the mesodermal bed of the cloaca.

12·5-*mm. embryo*

This embryo was obtained during operation for a ruptured ectopic pregnancy, the history of acute haemorrhage suggesting the recent death of the foetus.



Text-fig. 5. Embryo 12·5 mm. Description in text. A. Reconstruction of ventral surface from septum transversum to cloacal membrane. × *circa* 12. B. Reconstruction of median sagittal section of same. × 10. Dense mesoderm—black; ventral cloaca and allantois—large dots; cloacal membrane—horizontal lines; blood vessels—oblique lines; liver—circles. *A* = section 675 at the level of the cranial attachment of body stalk; *B* = section 775; *C* = section 825; interval between *B* and *C* is the extension of the coelom into the stalk and occupied by midgut loop; *D* = section 875 at the level of caudal attachment of the stalk; *G.t.* = section 888 at the level of the genital tubercle.

It is the first of the series in which the genital tubercle appears, and the small area between the tubercle and the stalk may be said to be the commencement of a true infra-umbilical portion of the abdominal wall.

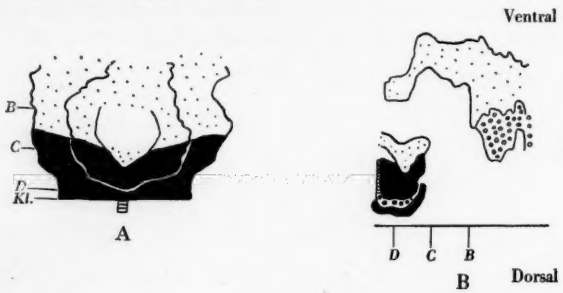
Section 864 (Pl. VI, fig. 23) shows the mesodermal condensation in the lower portion of the stalk and caudal to the vessels. It is becoming more marked and extends laterally to meet the paraxial downgrowth into the abdominal wall.

The relations can be followed from the reconstructions of the ventral wall (Text-fig. 5A) and a median sagittal section (Text-fig. 5B). The condensation can be traced caudally as it passes round the cloacal membrane to fuse with the general mesoderm on each side of the cloaca. It forms the basis of the genital tubercle which carries the membrane on its lower surface. The "infra-umbilical" area extends over eight sections measuring therefore about 0·08 mm.

13-*mm. embryo*

Obtained following abortion, this embryo, despite its greater size, is probably an earlier stage than the preceding. There is no genital tubercle and the tissues of the specimen show pathological changes.

The mesodermal condensation in the caudal portion of the stalk is, however, evident, and reference to the graphic reconstruction (Text-figs. 6A, B) shows again the continuation of this tissue down to and round the cloacal membrane. The allantoic end of the membrane itself is separated by a short interval from the stalk attachment.



Text-fig. 6. Embryo 13 mm. × *circa* 12. A. Reconstruction of lower ventral surface. B. Reconstruction of median sagittal section of ventral wall. Cloacal membrane—horizontal lines; dense mesoderm—black; ventral cloaca and allantois—large dots; liver—circles; *B* = section 910; *C* = section 954; interval between *B* and *C* is the extension of the coelom into the stalk and occupied by the midgut loop. *D* = section 1002 at the level of caudal attachment of the body stalk. *Kl.* = cloacal membrane.

14-*mm. embryo*

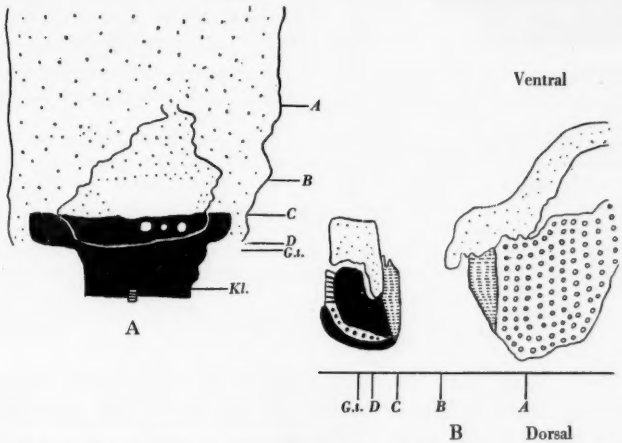
Obtained at operation and fixed while still transparent. Before fixation this embryo measured 15·5 mm. The sections were cut transversely.

The sections cranial to the stalk and intestinal loop show the ventral downgrowths from the paraxial mesoderm. These downgrowths exhibit some tendency to divide into strata foreshadowing the muscular layers of the anterior abdominal wall (Pl. VII, fig. 24). Ventrally the wall is completed by mucoid tissue.

Caudally the downgrowths from the paraxial mesoderm become less marked until they ultimately disappear entirely, but ventrally the mucoid tissue merges into a mesodermal condensation which in the lower sections is

well marked, and at the level of the caudal attachment of the stalk appears as a thick band of tissue on the superficial aspect of the allantoic vessels (Pl. VII, fig. 25). More caudally the band is continuous with the genital tubercle. The extent and relations of the mesodermal mass are shown in the graphic reconstructions (Text-figs. 7A, B).

The sections show clearly that the mesodermal condensation of the ventral portion of the infra-umbilical abdominal wall is not derived from the paraxial downgrowths, but has a separate origin. This part of the abdominal wall, as represented by the interval between the attachment of the stalk and the genital tubercle, has increased in length as compared with the 12·5-mm. embryo.



Text-fig. 7. Embryo 14 mm. × 10. A. Reconstruction of the ventral surface caudal to the septum transversum. B. Reconstruction of a median sagittal section of ventral wall. Dense mesoderm—black; cloaca and allantois—large dots; cloacal membrane—horizontal lines; liver—circles; blood vessels—interrupted horizontal lines. *A* = section 872 at the level of cranial attachment of the stalk. *B* = section 972; *C* = section 1044; interval between *B* and *C* is the extension of the coelom occupied by the midgut loop. *D* = section 1052 at the level of caudal attachment of stalk; *G.t.* = section 1060 at the level of genital tubercle. *Kl.* = cloacal membrane.

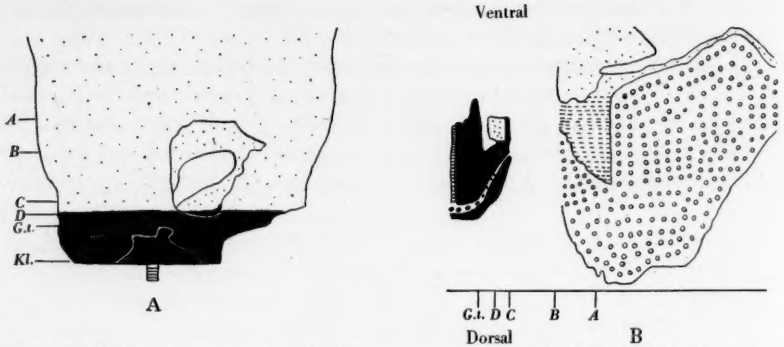
16·1-*mm. embryo*

Obtained following abortion.

The sections show the downgrowths from the paraxial mesoderm dividing into three strata.

These persist into the lower portion of the abdominal wall. In the upper sections (Pl. VII, fig. 26) the downgrowths reach about half-way round, but below the stalk they become continuous with the mesodermal thickening which commences on the caudal aspect of the stalk, and inferiorly forms the genital tubercle (Pl. VII, fig. 27). As can be seen from the graphs (Text-fig. 8A, B) this mesoderm forms the increasing infra-umbilical abdominal wall, the

genital tubercle, and the tissue between the cloaca and the ventral surface. The cloacal membrane, represented by a deep cleft, is on the caudal aspect of the genital tubercle.

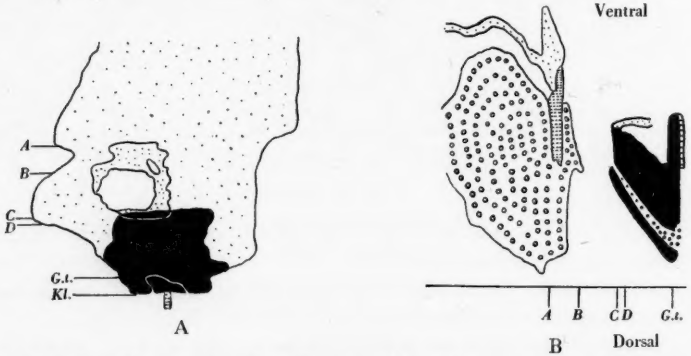


Text-fig. 8. Embryo 16·1 mm. × *circa* 12. A. Reconstruction of the ventral surface below the septum transversum. B. Reconstruction of a median sagittal section of ventral wall. Dense mesoderm—black; cloacal membrane—horizontal lines; ventral cloaca and allantois—large dots; liver—circles; blood vessel—interrupted horizontal lines. *A* = section 1148 at the level of the cranial attachment of the body stalk. *B* = section 1200. *C* = section 1256. *B–C* = extension of the coelom occupied by the midgut loop. *D* = section 1272 at the level of the caudal attachment of the stalk. *G.t.* = section 1292 at the level of the genital tubercle. *Kl.* = cloacal membrane.

23-*mm. embryo*

No history.

The downgrowths from the dorsal mesoderm are clearly divisible into three strata uniting in front. Above the body stalk the ventral junction is some



Text-fig. 9. Embryo 23 mm. × *circa* 12. A. Reconstruction of the ventral surface below the septum transversum. B. Reconstruction of a median sagittal section of ventral wall. Dense mesoderm—black; cloacal membrane—horizontal lines; ventral cloaca and allantois—black dots; liver—circles; blood vessels—interrupted horizontal lines. *A* = section 825 at the level of the cranial attachment of body stalk. *B* = section 857; *C* = section 913. Between *B* and *C* is the extension of the coelom occupied by the midgut loop. *D* = section 921 at the level of the caudal attachment of the stalk. *G.t.* = section 977 at the level of the genital tubercle. *Kl.* = cloacal membrane.

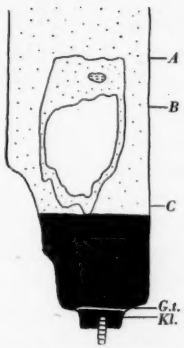
distance from the midline where the body wall is composed of tissue of loose texture (Pl. VIII, fig. 28). At the level of the body stalk the thickening forming the rectus muscle is visible; the splitting to form its sheath can be discerned. Below the body stalk the rectus sheath runs into a mesodermal mass forming the midventral wall (Pl. VIII, fig. 29). The infra-umbilical abdominal wall has increased considerably from that of the 16·1 embryo.

The mesodermal mass forms not only the ventral portion of the abdominal wall but the entire thickness of tissue superficial to the allantois and bladder (Text-fig. 9A, B).

30-*mm. embryo*

Obtained following abortion; sections cut longitudinally.

The graph (Text-fig. 10) shows the extent of the mesoderm and Pl. VI, fig. 22, is a section almost through the midsagittal plane, demonstrating the continuity of the stalk mesoderm with that forming the lower abdominal wall and genital tubercle.



Text-fig. 10. Embryo 30 mm. ×*circa* 12. Reconstruction of the ventral surface below the septum transversum. Dense mesoderm—black; cloacal membrane—horizontal lines; blood vessel—interrupted horizontal lines. *A* = cranial attachment of body stalk; between *B* and *C* is the extension of the coelom occupied by the midgut loop; *D* = the caudal attachment of the stalk; *G.t.* = genital tubercle. *Kl.* = cloacal membrane.

40-*mm. embryo*

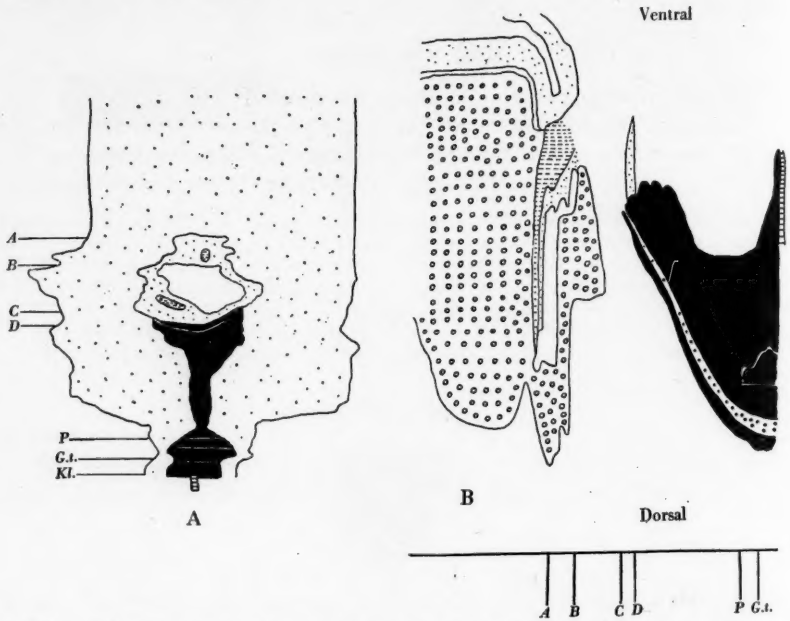
No history.

The ventral wall again shows the three muscular sheets terminating in the rectus in front.

The increasing thickness of the abdominal wall, well marked in this embryo, appears to be due to the somatopleure mesoderm rather than the paraxial myogenic downgrowths. The umbilical cord appears to be sunk in a depression in the anterior abdominal wall (Pl. VIII, fig. 30), which may be caused by a failure of the thickening to reach the midventral line owing to fixation at the umbilicus. The interval between the recti decreases in the more caudal sections.

The mesodermal mass in the midventral portion of the infra-umbilical wall, now of considerable length, is reinforced by extensions from the rectus sheath. It is also increased in thickness, stretching from the surface to the bladder and allantois internally.

In this embryo the condensations forming the pubic portions of the pelvis are present (Pl. VIII, fig. 31), and it is apparent that these arise from the central mesodermal mass (see graphs—Text-figs. 11A, B). Caudally the mesoderm forms the genital tubercle.



Text-fig. 11. Embryo 40 mm. × *circa* 12. A. Reconstruction of the ventral surface below the septum transversum. B. Reconstruction of a median sagittal section of ventral wall. Dense mesoderm—black; cloacal membrane—horizontal lines; ventral cloaca and allantois—black dots; liver—circles; blood vessels—interrupted horizontal lines. *A* = section 1152 at the level of the cranial attachment of body stalk; *B* = section 1184; *C* = section 1236; between *B* and *C* is extension of coelom; *D* = section 1252 at the level of the caudal attachment of stalk; *G.t.* = section 1408 at the level of the genital tubercle; *Kl.* = cloacal membrane; *P* = section 1384 at the level of the pubic condensations.

DISCUSSION

In the earliest embryo studied (the McIntyre I of 1·4 mm.) there is present a band of mesodermal tissue passing from the caudal aspect of the stalk round the cloacal membrane to the region of the termination of the primitive streak.

From the graphic reconstructions of the various stages up to that of 40 mm. the growth and development of this band of tissue can be traced; it is apparent

that from it are formed the structures in the midventral line of the infra-umbilical part of the body wall including the abdominal wall itself, the genital tubercle, cloacal fold, symphysis pubis, and the muscular coat of the bladder.

At the body stalk this mesoderm envelops the caudal aspect extending only a short distance along the stalk and surrounding not only the proximal portion of the allantois and allantoic vessels but furnishing a distinct band of tissue on the deep surface of the body wall caudal to the umbilicus in which are placed these structures; it becomes continuous with the mesoderm round the cloaca.

The more dense character of the tissue on this portion of the stalk as compared with the looser texture and mucoid appearance of the remainder is particularly well marked up to the 23-mm. stage. In the 30 and 40 mm., while still apparent, it does not so sharply contrast with the surrounding tissue in the stalk or upper half of the abdominal wall, as though its strength were becoming exhausted by a "paying-out" to its derivatives.

The question of the origin of the umbilical part of the mesoderm has already been discussed and the conclusion formed that it arises from the primitive mesoderm representing the site of the posterior mesodermal field of the embryonic shield. Extending as wing-like processes round the cloacal membrane it becomes continuous with the secondary mesoderm from the end of the primitive streak.

The observations leading to the assumption that the primitive streak terminates at or even before the commencement of the cloacal membrane are recorded earlier, and the reasons given for discarding the suggestion of Keibel and others that the cloacal membrane itself is a modification of the primitive streak which may extend as far as or even on to the body stalk.

The first indication of the existence of a body wall below the attachment of the stalk is in the 12·5-mm. embryo, where the interval between the genital tubercle and the stalk is occupied by the mesodermal mass under discussion. This also shows lateral extensions passing some distance round the body wall and on each side of the genital tubercle forming the cloacal folds.

The relative decrease in the breadth of attachment of the body stalk, with the consequent ventral encroachment of the paraxial downgrowths into the somatopleure in the older embryos, minimizes the actual area of abdominal wall formed by this mesoderm, but it can be observed in its position in the midventral line up to the 40-mm. stage and is probably ultimately represented in the adult body wall by the symphysis pubis, lower part of the rectus sheath and infra-umbilical portion of the linea alba.

The extension of the 'coelom' into the pelvis follows a 'taking in' of the stalk into the body of the embryo with a prolongation of the 'umbilical coelom' (i.e. that part of the body cavity passing into the stalk) between the hindgut and the ventral wall containing the allantois and the vessels. This can be seen in the reconstruction of the 4·5- and 7-mm. embryos. The mesoderm of the caudal end, in which is embedded the cloaca, remains unsplit, and thus that

portion which passes from the hind end of the primitive streak to become continuous with the downgrowth from the stalk is equivalent to somatopleure and splanchnopleure, forming not only part of the parietes, but the fibrous and muscular coats of the bladder. The condensations forming the pubes seen in the 30- and 40-mm. embryos are obviously arising in the midline from the mesoderm in this region.

ECTOPIA VESICAE AND EPISPADIAS

Any investigation of the embryology of this region inevitably involves the problem of ectopia vesicae and epispadias and the plethora of suggestion—much of it empirical, most of it unsatisfactory—which has been offered to account for this congenital defect.

On the question of the aetiology of extroversion of the bladder and epispadias most surgical text-books are content with the statement that the deformity can be ascribed to arrested development of the lower abdominal parietes. Others elaborate ingenious mechanical theories to account for the condition, for example, "pressure of the umbilical cord which passes between the lower limbs of the foetus to a dorsally placed placenta" (*Surgery of Childhood*, J. Fraser); or again, "Epispadias is really a hypospadias in which torsion of the penis has occurred at an early stage of foetal life so that the under surface becomes the upper" (*System of Surgery*, Choyce, vol. II, 1932).

For some time the views of Keibel were very generally accepted. According to this author the cloacal membrane should be regarded as a modified part of the primitive streak and ectopia vesicae as a persistent portion of the blastopore.

Keibel and Elze believe "that the primitive streak extends along the body stalk, as in some sections the ectoderm covering the body stalk shows a local thickening which is nearly in contact with the allantoic duct". The description is more akin to that of the cloacal membrane becoming obliterated by the incursion of mesoderm than to that of a primitive streak.

Keith agrees with Berry Hart that the primitive streak forms the post-umbilical wall and suggests that extroversion of the bladder is an unclosed condition of the streak. Should this be so, is there any reason why the non-closure of the blastopore should be confined to the allantoic portion of the ventral wall of the cloaca? Would it not be reasonable to assume that amongst the many recorded cases of this condition a few would show more extensive or additional defects involving perhaps the anal region or some degree of spina bifida which, according to Grosser (1927), is a non-fusion of the lips of the primitive groove? Or, again, one would expect to find the condition in lower mammals where the primitive streak forms not only the secondary but the primary mesoderm.

Cogent reasons have earlier been advanced for the rejection of the averment that the primitive streak extends on to the body stalk. There is neither

histological nor ontogenetical evidence to support the suggestion that the cloacal membrane is a modified primitive streak. The facts I have recorded as observed in McIntyre I, viz., that the primitive streak terminates at or even some distance before the commencement of the cloacal membrane, is in agreement with most of the recent work already referred to.

Keibel (1896) later retracts from his original position, and, having observed some diminution in the absolute length of the cloacal membrane in embryos of about 6 mm., is of the opinion that ectopia vesicae may result from a failure of shortening of the membrane at this stage and accordingly is of a later developmental period than was presumed in his previous observations.

Enderlen (1908) states that if such assumptions were correct, then the cloacal membrane must extend up to the body stalk, and in his opinion this is actually so up to the 9-mm. stage; the earlier the error occurs the more complete the defect, ranging from complete extroversion to hypospadias.

In the embryos 4·5 and 7 mm. of this series the cloacal membrane did not reach the body stalk, and similar observations on even earlier stages have been recorded.

Felix (1911) found that the cloacal membrane reaches the body stalk up to 18 somites.

From the study of this region in a series of embryos ranging from 1·4 to 40 mm. the following facts have been observed and are recounted graphically in the drawings.

Firstly, in the presomite stage the cloacal membrane involves not only the cloaca, but exists as an area of contact between the amniotic ectoderm and allantoic endoderm. This is seen in section 119 of the McIntyre embryo and has been recorded by a number of observers including Florian (1930) in embryo Bi II and Sternberg (1927) in an embryo of 4 somites. The cloacal membrane is therefore at this stage relatively extensive reaching on to and along the proximal end of the connecting stalk. In the McIntyre embryo the "allantoic cloacal membrane" extends only over one section, and in the intervening area between that and the hindgut cloacal membrane, the ectoderm and endoderm are separated by mesoderm. In the Sternberg embryo there are three separate portions of the allantoic cloacal membrane, and two in the Bi II of Florian. In the next embryo studied of 2·4 mm. the cloacal membrane is limited to the cloaca proper, with a mesodermal interval between its allantoic extremity and the stalk.

It is therefore reasonable to assume that the interrupted contact of allantoic endoderm and ectoderm is evidence of the disappearance of the allantoic cloacal membrane, and not the commencement or extension of the existing cloacal membrane, and that, primarily, the "membrane" was an area of contact of ectoderm and endoderm extending from the hindgut cranially,[1] and caudally terminating some distance along the stalk.

[1] The terms "cranially" and "caudally" are here descriptive of the relations existing before the appearance of the tail fold.

The McIntyre I thus represents a stage showing the process of obliteration of the "allantoic cloacal membrane". Florian (1930) observed signs of degeneration in this part of the membrane.

Examination of the sections of this embryo leads to the conclusion that the separation of the ectoderm and endoderm in this region is effected by the mesoderm growing in towards and ultimately fusing in the midline. This mesoderm seems to be derived from the wing-like processes arising from the hind end of the primitive streak extending round on either side of the chordal portion of the cloacal membrane, and encroaching on the midline towards the allantoic end. One would therefore anticipate that at a later stage there would be evidence of a decrease in size and extent of the membrane, and a study of Table I discloses such a condition occurring in embryos of about 6–8 somites. It is of interest that the actual obliteration is in progress during what is probably the period of maximum activity of the primitive streak and when one would predict an optimum mesodermal energy.

In the 2·4 mm. and more definitely in the 4·5 mm. the cloacal membrane is placed some distance behind the caudal attachment of the stalk and is limited to the ventral aspect of the cloaca.

The processes of mesoderm encircling the membrane and fusing with the mesenchyme of the stalk form the band of denser tissue whose development has been followed up to the 40-mm. embryo. The extent covered by structures arising from this mesodermal tissue is indicated in the reconstructions by the areas shown in black from which it becomes evident that failure of this tissue to develop would result in the persistence of the original extensive cloacal membrane which would form the infra-umbilical portion of the ventral abdominal wall, the absence of the genital tubercle with defective formation of the external genitals, and deficiency of the symphysis pubis. With the breaking down and absorption of the cloacal membrane which normally occurs about the second month, the condition of ectopia vesicae would then be established. Minor mesodermal failure would be responsible for the lesser defect of epispadias.

The mesodermal septum dividing the cloaca into ventral and dorsal parts reaches the surface separating the anal from the urogenital orifice so that the extroversion is limited to the ventral cloaca, and the anal region develops normally.

As the mesodermal band has been shown to consist of primary and secondary mesoderm, the fault might be apportioned to the one or the other, or again could be accounted for by an undue adherence of amniotic ectoderm and allantoic endoderm, what Rossenbeck (1923) would term an "incomplete loosening" of the germinal layers preventing the mesodermal inroads.

Bearing in mind the role of the primitive streak and that it is less productive in the human than in any other form, one inclines to the view that the aetiological factor is deficiency and non-development of the mesodermal processes which normally arise from the hind end of the primitive streak, extend

on either side of the cloacal membrane, press in towards the midline, obliterate its allantoic extension, and, continuing on to the stalk, merge into the primary mesoderm.

Sternberg (1926), in a study of the cloacal membrane of young embryos, concludes that the period of shortening of the membrane coincides with the appearance of the first somites, and that therefore extroversion must occur before the degeneration of the allantoic cloacal membrane. He states of the condition: "Die teratogenetische terminationperiode der Bauchblasenspalte und der mit ihr verwandten missbildungen ist demnach wesentlich früher, als man bisher angenommen hatte und zwar etwa in jenes stadium zu verlegen im welchem sich die ersten Ursegmente ausbilden."

Extroversion of the bladder is a condition peculiar to the human species (Kermauner, 1909). This is probably due to the precocious development of the allantois, e.g. the Peh-Hochstetter embryo (Rossenbeck, 1923), in accordance with the mode of nutrition in the human, coupled with the diminished activity of the primitive streak. In *Tarsius* (Hubrecht, 1909) the primitive streak thickening appears as a forward extension of the proliferating caudal margin of the embryonic shield from which is formed the mesoderm of the connecting stalk, whereas in the human the origin of the primitive streak is much more cranial.

The "allantoic cloacal membrane" appears to be a human characteristic (Sternberg) and is possibly permitted by the more cranial origin of the streak with the consequent lessened mesodermal production at this point. Again, the extension of the cloacal membrane may be a secondary development due to the activity of the ectodermal cells of the amnion described by Florian (1930) which, encroaching on and destroying the mesoderm of the connecting stalk, thereby permits of the extension of the amniotic space at the expense of the stalk tissue.

The cloacal membrane appears at a relatively early stage in the human embryo (Fetzer-Florian embryo, 1930), and perhaps is not so much a development as an actual persistence of contact between the ectoderm and endoderm.

One concludes that ectopia vesicae is to be regarded as yet another of the long list of congenital defects to be laid to the account of the mesoderm and in some way occasioned by the declining activity of the primitive streak.

The determination of the causal factor effecting the mesodermal deficiency is a problem the answer to which may be found in the complicated mechanism of "organizers", the initiation of "Field Gradients" with their subtle interaction and susceptibility to adverse environmental conditions.

SUMMARY

1. A study has been made of a series of embryos ranging from 1·4 to 40 mm. in length, with graphic reconstructions, tracing the development of the infra-umbilical abdominal wall.

2. The infra-umbilical portion of the anterior abdominal wall, the genital tubercle, symphysis pubis, and muscular coat of the bladder, are formed from a well-defined band of mesoderm.

3. This band of mesoderm has a twofold origin: (*a*) from the caudal margin of the embryonic shield—primary mesenchyme; (*b*) processes of secondary mesoderm passing round the cloacal membrane from the hind end of the primitive streak.

4. In the embryo McIntyre I the cloacal membrane is in two parts, one of which implicates the allantoic diverticulum.

5. At an early stage the cloacal membrane is a relatively large area of contact of ectoderm and endoderm extending some distance along the allantoic diverticulum.

6. The allantoic cloacal membrane is later obliterated by the mesoderm pressing in towards the midventral line between the ectoderm and endoderm.

7. Extroversion of the bladder is due to mesodermal deficiency, particularly of the processes of secondary mesoderm arising from the hind end of the primitive streak, following on which there is persistence of the primary extensive cloacal membrane, impaired development of the muscular coat of the bladder, of the symphysis pubis, and of the formation of external genitals and infra-umbilical portion of anterior abdominal wall.

8. Epispadias is a similar mesodermal error in a minor form.

My thanks are due to my Chief, Prof. Blair, for his helpful criticism and valuable suggestion, and to Dr Norman H. W. Maclaren for his very generous assistance and advice.

The drawings of the wax plate reconstructions were made by Mr Alexander Rettie of this Department.

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EXPLANATION OF PLATES I–VIII

Plate I

Fig. 1. Photograph of a drawing of a wax plate reconstruction of embryo McIntyre I. ×*circa* 40. The arrows indicate the plane of the graphic reconstruction (Text-fig. 1B). The numbers refer to the sections limiting the area reconstructed in the graph.
Fig. 2. Section 84 of 2·4-mm. embryo. Photomicrograph ×*circa* 80. *M.* = mesodermal condensation at the caudal attachment of the stalk.
Fig. 3. Section 146 of embryo McIntyre I. Photomicrograph ×*circa* 140. Description in text. *Y.* = cavity of the yolk sac.
Fig. 4. Section 143 of embryo McIntyre I. Photomicrograph ×*circa* 140. Description in text. *Y.* = cavity of the yolk sac.

Plate II

Sections of embryo McIntyre I. Photomicrographs ×*circa* 140. Description in text.
Fig. 5. Section 130. *Y.* = cavity of yolk sac. *a.e.c.* = amnio-embryonic cavity.
Fig. 6. Section 129. *Y.* = cavity of yolk sac. *a.e.c.* = amnio-embryonic cavity.
Fig. 7. Section 125. *Y.* = cavity of yolk sac. *a.e.c.* = amnio-embryonic cavity.
Fig. 8. Section 122. A process of ectoderm can be seen extending up to yolk sac endoderm but separated from it by a small interval in which are mesodermal cells. *Y.* = cavity of the yolk sac. *a.e.c.* = amnio-embryonic cavity. *A.* = allantoic diverticulum.

Plate III

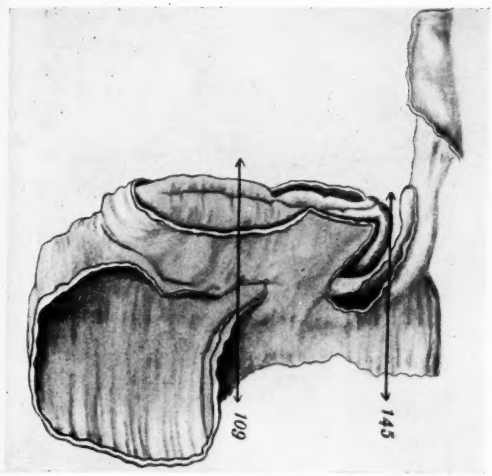
Figs. 9–11 are sections of embryo McIntyre I. Photomicrographs ×*circa* 140. Description in text.
Fig. 9. Section 119. *Y.* = cavity of yolk sac. *a.e.c.* = amnio-embryonic cavity. *A.* = allantoic diverticulum.
Fig. 10. Section 118. *Y.* = cavity of yolk sac. *a.e.c.* = amnio-embryonic cavity. *A.* = allantoic diverticulum.
Fig. 11. Section 112. *Y.* = cavity of yolk sac.
Fig. 12. Photograph of a drawing of a wax plate reconstruction of embryo 2·4 mm. in length. ×*circa* 40. The arrows indicate the plane of the graphic reconstruction (Text-fig. 2B). The numbers refer to the sections limiting the area reconstructed in the graph.

Plate IV

Sections of embryo 4·5 mm. (McIntyre II). Photomicrographs ×*circa* 110. Description in text.
Fig. 13. Section 136. *M.* = mesodermal condensation.
Fig. 14. Section 131. *Kl.* = cloacal membrane.
Fig. 15. Section 138. *M.* = mesodermal condensation.
Fig. 16. Section 144.

Plate V

Fig. 17. Photograph of a drawing of a wax plate reconstruction of a 7-mm. embryo. ×*circa* 12. The arrows indicate the plane of the graphic reconstruction (Text-fig. 4B). The numbers refer to the sections limiting the area reconstructed in the graph.
Figs. 18–20 are sections of embryo 7 mm. in length. Photomicrographs ×*circa* 120. Description of sections in text.
Fig. 18. Section 329. *Kl.* = cloacal membrane.
Fig. 19. Section 373. *M.* = mesodermal condensation.
Fig. 20. Section 345. *Kl.* = cloacal membrane.



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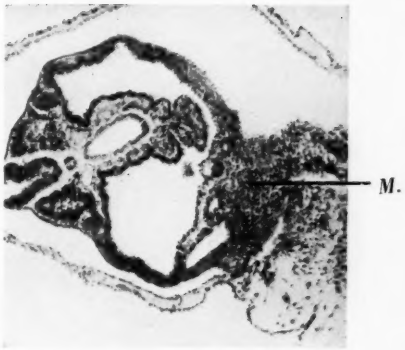

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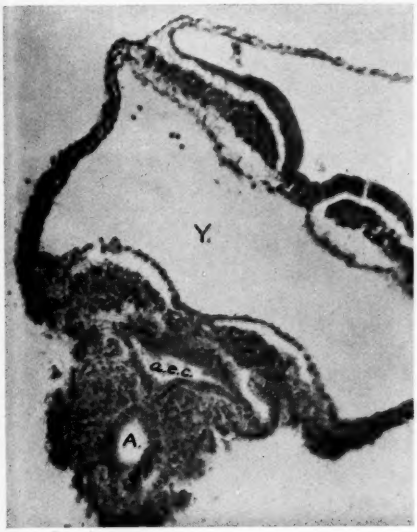

Fig. 9.

Fig. 10.

Fig. 11.

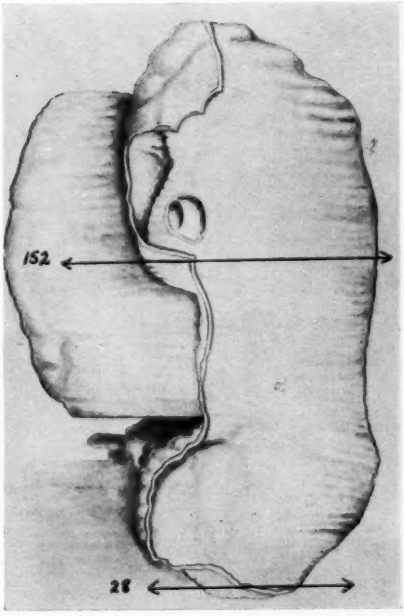

Fig. 12.

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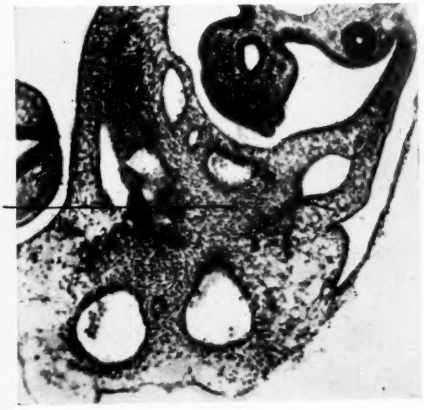
Fig. 13.

Fig. 14.



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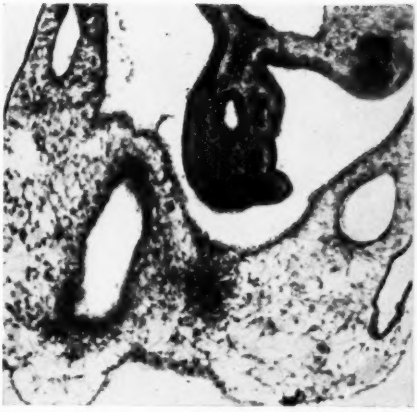

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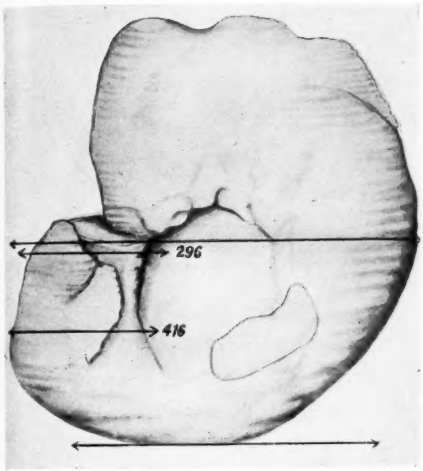
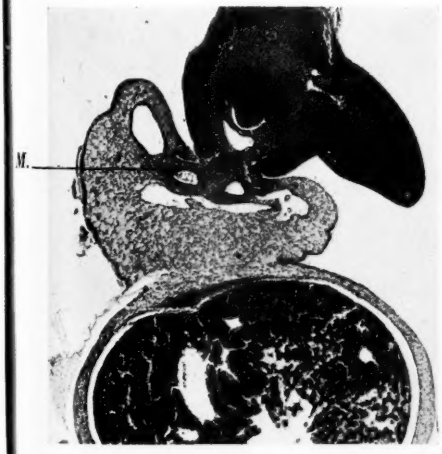


Fig. 17.

Fig. 18.



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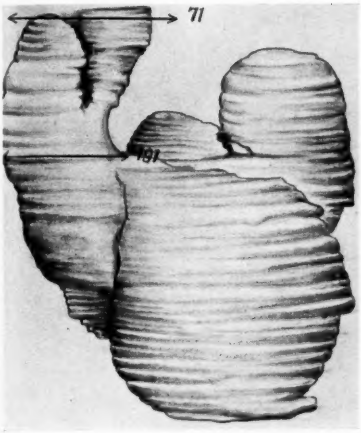

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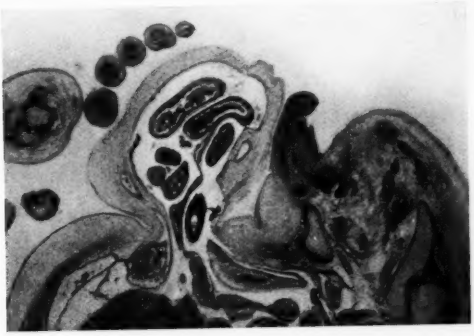

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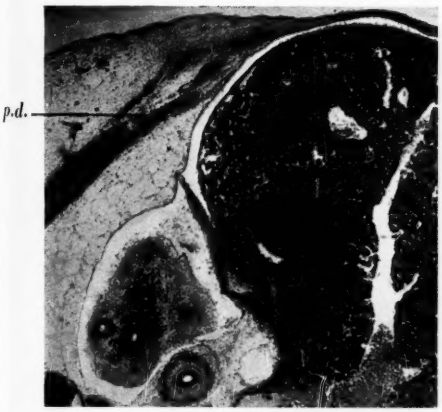

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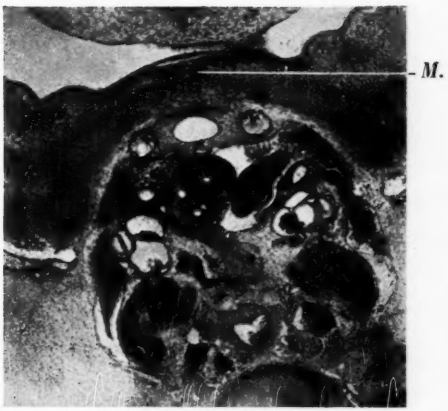

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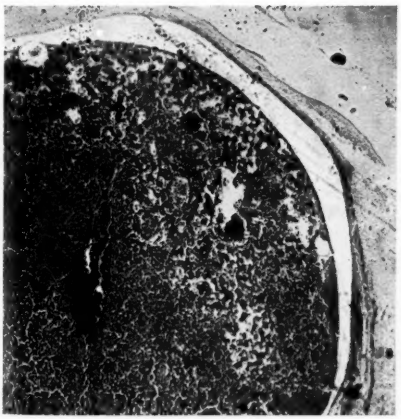

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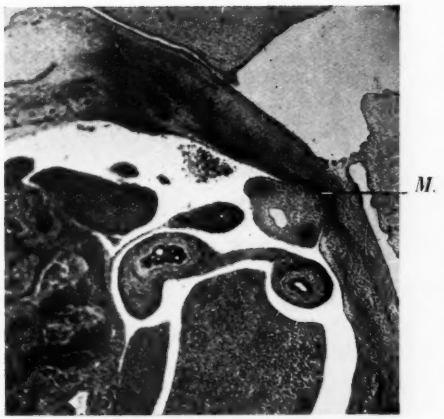

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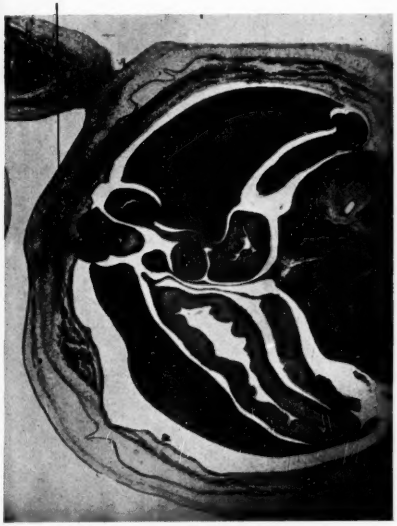

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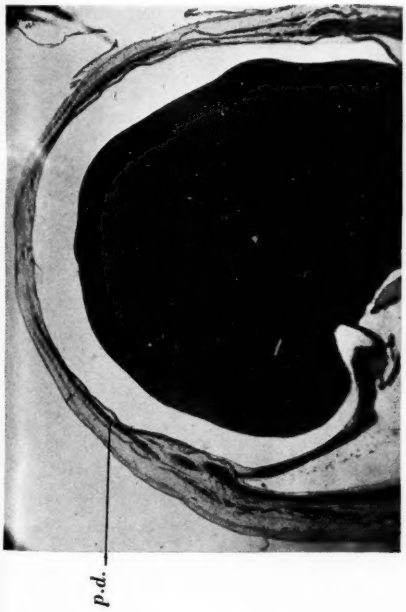

Fig. 28.

Fig. 30.

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PLATE VI

Fig. 21. Photograph of a drawing of a wax plate reconstruction of a 4·5-mm. embryo (McIntyre II). ×*circa* 18. The arrows indicate the plane of the graphic reconstruction (Text-fig. 3B). The numbers refer to the sections limiting the area reconstructed in the graph.

Fig. 22. Longitudinal section of a 30-mm. embryo. Photomicrograph ×*circa* 7.

Fig. 23. Section 864 of 12·5-mm. embryo. Photomicrograph ×*circa* 27. Description in text. *M.* = mesodermal condensation.

PLATE VII

Fig. 24. Section 761 of a 14-mm. embryo. Photomicrograph ×*circa* 17. Description in text. *p.d.* = paraxial myogenic downgrowth.

Fig. 25. Section 1081 of a 14-mm. embryo. Photomicrograph ×*circa* 17. Description in text. *M.* = mesodermal condensation.

Fig. 26. Section 1079 of a 16·1-mm. embryo. Photomicrograph ×*circa* 17. Description in text.

Fig. 27. Section 1278 of a 16·1-mm. embryo. Photomicrograph ×*circa* 17. Description in text. *M.* = mesodermal condensation.

PLATE VIII

Fig. 28. Section 773 of a 23-mm. embryo. Photomicrograph ×*circa* 7. Description in text. *p.d.* = paraxial myogenic downgrowth.

Fig. 29. Section 924 of a 23-mm. embryo. Photomicrograph ×*circa* 7. Description in text. *M.* = mesodermal condensation.

Fig. 30. Section 1216 of a 40-mm. embryo. Photomicrograph ×*circa* 7. Description in text.

Fig. 31. Section 1408 of a 40-mm. embryo. Photomicrograph ×*circa* 7. Description in text. *s.p.* = condensation forming the pubes.

THE INNERVATION OF THE PERIODONTAL MEMBRANE OF THE CAT, WITH SOME OBSERVATIONS ON THE FUNCTION OF THE END-ORGANS FOUND IN THAT STRUCTURE

By W. LEWINSKY and D. STEWART

From the Department of Anatomy, Manchester University

In a recent paper, the authors gave an account of an investigation which they had made into the innervation of the periodontal membrane in man. They were able to show that the nerve fibres in this tissue broke up into arborizations, many of which ended in round knob-like bodies (4). The results obtained were similar to those which have been described by Kadanoff (3), who also used human material, and were not unlike the findings of Bradlaw (1) who based his conclusions on the periodontal membrane of the monkey. They, however, differed markedly from the findings of van der Sprenkel (5) who, in his examination of the periodontal membrane of the young mouse, had seen an entirely different type of nerve ending. The authors put forward the suggestion in this paper that the discrepancies in van der Sprenkel's results which differed so markedly from those of all other investigators in this field, might be due to the different material which he employed.

The present investigators considered that the problem was of sufficient importance to merit a more extensive study, and they have therefore widened the field of their investigation with the intention of studying the innervation of some typical examples of the more readily obtainable mammalian genera. The work on the cat has been completed, and the results which have been obtained are of such interest as to deserve a further communication on this subject before the rest of the work is completed.

The jaws were removed from freshly killed cats, and the teeth with their alveolar plates attached were cut away with a fret-saw. These were fixed in ammoniated alcohol, decalcified in 5 per cent nitric acid and impregnated by the usual Cajal technique. They were then embedded in paraffin and cut in serial sections. When the specimens were examined, the general arrangement of the nerve fibres corresponded with what had been found in the human, that is to say, the nerve fibres ran from the apex towards the gum in bundles in the more peripheral part of the periodontal membrane. These longitudinally running bundles were reinforced at intervals by other fasciculi which entered the periodontal membrane from the alveolar plate. An interesting observation was made regarding the distribution of these latter fasciculi. It is generally believed that these fasciculi, after passing through the alveolar plate and entering the periodontal membrane, turn and run towards the gingival margin. In the cat,

however, it was found that very frequently this did not occur. The bundle, on entering the periodontal membrane, divided into two parts; one followed the usual course and passed towards the gingival margin, while the other turned in the opposite direction and ran towards the apex of the tooth (Pl. I, fig. 1). As far as we know, this division of the alveolar accessory nerves into two parts has not been described before. With the knowledge we had thus obtained from the cat, we re-examined our human slides and were able to find specimens there which show a similar arrangement of the nerve fibres. Although nothing definite seems to be known, it appears to be generally accepted that the larger proportion of the nerve fibres to the periodontal membrane comes from the apical region, and that the fibres which enter from the alveolus are of relatively less importance. Our examination of the periodontal membrane of the cat, however, suggests that these latter fibres play a much greater part in the innervation of the periodontal membrane than has hitherto been imagined.

When the nerves in the periodontal membrane were examined, whatever their source, it was found that they consisted of two different types, coarse and fine fibres. The former were markedly thicker but were less heavily impregnated than the latter and had a dark brown appearance while the finer fibres were very often stained intensely black. The most striking difference between them, however, was the way in which the nerve fibres ended. The main nerve bundles lay in the peripheral part of the periodontal membrane, and the coarse fibres always remained in the outer part of that tissue. The finer fibres, however, turned inwards and broke up into fine ramifications in the more medial part of the membrane (Pl. I, fig. 2). Although in several of our specimens the impregnation was extremely good and the finest fibrils were clearly seen, in no instance were we able to trace any of these fibres into the cementum. The coarser fibres terminated in an entirely different manner, in special end-organs (Pl. I, fig. 3). These end-organs were spindle-like in shape and were formed by the nerve fibre becoming twisted like a spiral spring. At intervals on the convolutions there were rounded thickenings. From their position in the peripheral half of the periodontal membrane and their shape, they bore a certain resemblance to van der Sprenkel's second type of nerve ending, namely the terminal reticula around the nuclei of the connective tissue of the peripheral layer of the membrane. As figured in van der Sprenkel's diagram, these end-organs are too small to determine their structure clearly, but they seem to form a definite network. It will therefore be seen that although, as has been said, in shape and position they correspond with the end-organs which we have found on the coarse fibres in the cat, yet structurally they differ from each other. The end-organ in the cat does not appear to have any special relationship to any particular cells, neither does it form a network but has a spiral-like course without any side branches or reticular endings.

This discovery of another type of nerve ending in the periodontal membrane of the cat, therefore, gives support to our earlier suggestion that there may be marked differences in the structure of the nerve endings in the periodontal

membrane in different types of mammals. This is confirmed by some observations which we have made in the rabbit. The technique used for the preparation of this material was identical with that used for the cat, and a few sections were completed for examination while this paper was being written. It has therefore been possible to make a few preliminary observations on the nerve supply of its periodontal membrane. Here again an entirely different type of nerve ending occurs on the coarse fibres (Pl. I, fig. 4). In the rabbit these fibres end in coarse, widely separated arborizations with thickened rounded endings which do not in any way resemble the end-organs in any other animal which has up to the present been examined.

The two types of nerves with their different distribution and terminations would suggest that they have different functions and probably transmit to the brain different types of nerve impulses. The existence of coarse, medium and fine fibres in the roots of the spinal nerve has been made known to us by such investigators as Ranson and Hess, who have established that the fine fibres probably carry painful stimuli and the coarse fibres sensations of pressure and touch. Recently, Woollard (8) has emphasized the significance of these fibres in connexion with his work on the nerve supply of the skin. Windle (7) has investigated this problem, as it affects the pulps of the teeth and the nerves to the root canals. He found that in the cat and the dog all the myelinated nerves were of small or medium size and that no large fibres were present. When he devitalized the teeth by removing the pulps he found that the majority of the reacting cells in the Gasserian ganglion were of the small or medium type which have been considered to be the cells for the finer or the medium fibres and therefore associated with the conduction of painful stimuli.

Brashear (2) repeated the work of Windle but at the same time widened the scope of the enquiry to include the fibres of the periodontal membrane. The results which he obtained from the pulp corresponded to those which Windle had seen, and in the cat he found no fibres more than 9 μ in diameter, although in the human some fibres were found which reached 10 μ. In the Gasserian ganglion, however, after removal of the pulp, about 10 per cent of the cells showing chromatolysis were of Clark's large cell type "A". The author points out that this reaction of the larger cells is probably of little significance and may be due to causes not associated with the removal of the pulp, such as the disturbance of the nutrition of the periodontal membrane. In the periodontal membrane, Brashear found that nerves of all sizes are present and that about 20 per cent of the fibres in this tissue are 10 μ or larger in thickness. From the work of Windle and Brashear, it can therefore be assumed that the nerve fibres of the pulp are of the small or medium-sized type and therefore only capable of transmitting painful stimuli, a characteristic of the pulp which is well known from clinical observations. On the other hand, as the large type of nerve fibre is also present in the periodontal membrane, it is to be presumed that the periodontal membrane is also sensitive to tactile and pressure stimuli as well as to painful ones. Several years ago, one of us (6) was able to show that

Fig. 1.

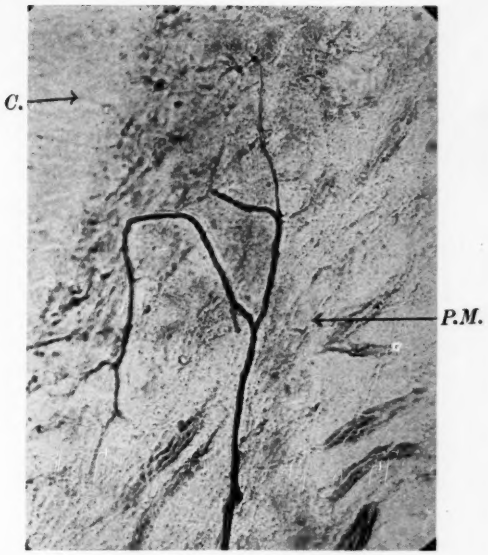

Fig. 2.

Fig. 3.

Fig. 4.

the teeth are able to react to quite low pressure stimuli and are able to localize such stimuli with a considerable degree of accuracy. The removal of the pulps of the teeth did not appear to alter the ability of the tooth to detect tactile stimuli, and the conclusion was therefore drawn that these stimuli must be conducted by means of the periodontal membrane.

The account given in the present investigation of the discovery of specialized end-organs in association with the coarse fibres, and fine terminal arborizations with the thinner fibres is therefore of importance as it gives a connecting link between the clinical investigations of Stewart on the functions of the periodontal membrane and Brashear's observations on the two types of nerve fibre which he found in that structure.

SUMMARY AND CONCLUSIONS

In the cat the innervation of the periodontal membrane is from two sources, (1) fibres running from the apical region and (2) fibres entering laterally from the alveolar plates. The latter divide into two fasciculi, those running towards the apex and those running towards the gingival margin. The alveolar nerve fibres appear to form a greater proportion of the nerve fibres to the periodontal membrane than has hitherto been believed. The nerve fibres in the periodontal membrane are of two types, (1) thick fibres confined to the peripheral part of the membrane which have specialized end-organs at their termination, (2) finer nerve fibres which pass to the deeper part of the periodontal membrane and break up into fine arborizations without terminal organs. It is suggested that the thick fibres with their specialized end-organs are associated with tactile and pressure sensations, while the finer fibres are associated with those of pain. The authors were unable to trace any fibres into the cementum.

ACKNOWLEDGEMENTS

We have to thank Prof. Stopford for his customary kindness in allowing us full facilities in the Anatomy Department. Mr Gooding is again responsible for the microphotographs with which this paper is illustrated, and we are greatly indebted to Miss Dobson for the assistance which she has given us in preparing this paper for the press.

EXPLANATION OF LETTERING

A. Alveolar bone.
C. Cementum.
P.M. Periodontal membrane.

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THE RELATIONS OF ENDOGENOUS AND EXOGENOUS FACTORS IN BONE AND TOOTH DEVELOPMENT

THE TEETH OF THE GREY-LETHAL MOUSE

By HANS GRÜNEBERG

Department of Zoology, University College, London

INTRODUCTION

The grey-lethal mutation in the mouse (Grüneberg, 1935, 1936 *a*, *b*) is characterized by a complex of symptoms which is unparalleled in human pathology. The fur lacks the yellow pigment. No tooth ever erupts. Without artificial feeding death invariably occurs between the 22nd and the 30th day of life. Liquid food prolongs the life for a fortnight or so, but does not materially change the condition. Death is thus not due to mechanical troubles in feeding, but its actual cause remains unknown.

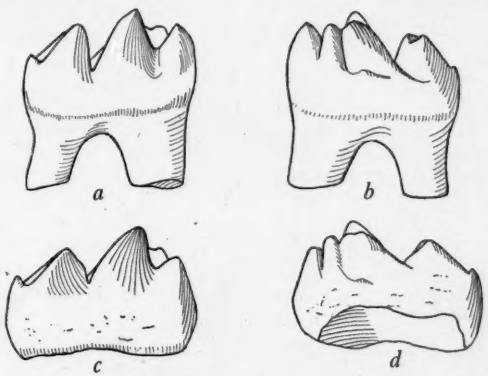
The main feature of the skeleton is the entire lack of all secondary absorption processes. Thus bone substance once laid down in development is preserved in its entirety. The geometrical proportions of bones can only be maintained during growth by secondary remodelling processes by which some of the bone temporarily laid down is eventually removed. This has been thoroughly worked out in detail by means of madder feeding in the pig by Brash (1934). If, however, these secondary modelling processes fail to act, very characteristic anomalies in bone shape are bound to develop. The grey-lethal bones correspond excellently to these expectations. There is, therefore, no doubt that the grey-lethal complex of symptoms is in fact largely due to a failure of the secondary resorption processes on the bones. In addition there is incomplete calcification, both in bone and dentine. This leads to failure of eruption of the teeth as has been described in detail elsewhere.

In this paper a more thorough description of the crown shape of the grey-lethal molars will be given. The anomalies to be described are constantly found. Generally, the grey-lethals are remarkably constant in the abnormal conditions they show: the differences between individuals other than those due to age are practically negligible. It will be discussed below whether and to what extent the anomalies in the shape of teeth can be explained in terms of the peculiarities already well established in the grey-lethal mouse. Finally, the bearing of these facts on the general conception of the action of exogenous stimuli and the response of the bones will be considered.

MORPHOLOGY OF MOLARS

For comparison, developmental stages of normal teeth must be used in which the shape of the cusps has not yet been altered by wearing off. The illustrations (Text-figs. 1–4) show camera lucida drawings of a 14 days old grey-lethal mouse and a normal litter-mate. The roots of first molars in the normal are still incomplete, while the uncalcified vestiges of roots in the grey-lethal, dried up and shrivelled and largely lost during the preparation, are here of no concern.

As already mentioned in the first paper (1935), the lower first molar is strongly compressed laterally in the grey-lethal. At the same time it is noticeably longer than the corresponding tooth of the normal. The median and lateral surfaces are considerably flattened, while those of the normal are bulging and bulbous. This applies particularly to the fissures between the

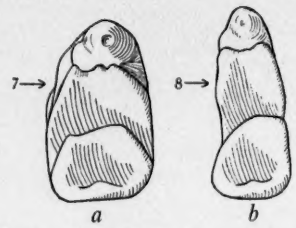


Text-fig. 1. Left lower first molar of grey-lethal (*c*, *d*) and normal litter-mate (*a*, *b*), 14 days old. *a* and *c*, median view; *b* and *d*, lateral view.

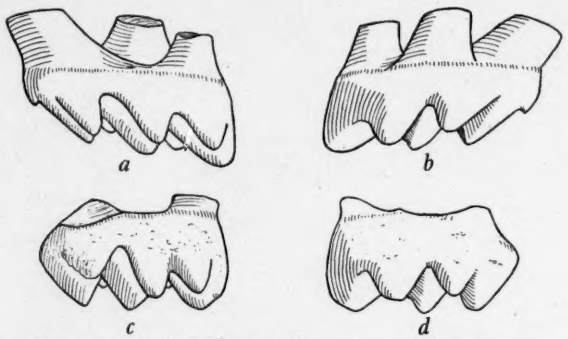
cusps, which are shallow grooves or impressions only, while they are deep and wide in the normal. The cusps themselves are a little more erect and project slightly more over the tooth neck. The flattened surfaces are not smooth and regular, but show minute and irregular depressions, the majority of which are directed parallel to the axis of the mandible. All these anomalies are more strongly developed on the lateral surface which as a whole is considerably lower. In lateral view a good deal of the inside cavity of the tooth is therefore visible (Text-figs. 1–2).

In the upper first molars, the most conspicuous anomaly is that all the cusps are erected, though to a varying extent. In a normal first upper molar, all the cusps have their axes practically parallel to each other, including angles of between 40 and 45° with the plane of the neck of the tooth. In the grey-lethal tooth the axes of the cusps have an inclination of between 55 and 75°. This difference is most clearly seen in lateral view, since the lateral surface is

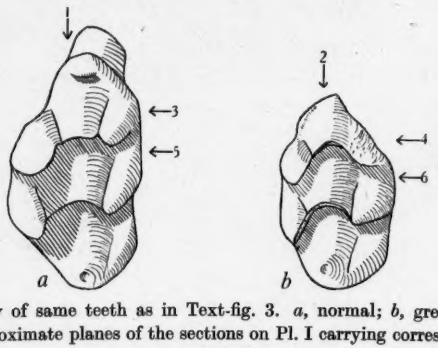
comparatively flat while the median surface in both types bulges towards the sagittal plane (Text-fig. 3). Comparison of such teeth strongly suggests that



Text-fig. 2. Top view of same teeth as in Text-fig. 1. *a*, normal; *b*, grey-lethal. The arrows indicate the approximate planes of the sections on Pl. I carrying corresponding numbers.



Text-fig. 3. Left upper first molar of grey-lethal (*c*, *d*) and normal litter-mate (*a*, *b*), 14 days old. *a* and *c*, lateral view; *b* and *d*, median view.



Text-fig. 4. Top view of same teeth as in Text-fig. 3. *a*, normal; *b*, grey-lethal. The arrows indicate the approximate planes of the sections on Pl. I carrying corresponding numbers.

pressure from the front has acted on the grey-lethal tooth so as strongly to erect the cusps.

Another striking difference is found in the outline of the crown when seen in top view (Text-fig. 4). Whereas the normal tooth bulges broadly towards

the middle line of the body, this protrusion is very much less pronounced in the grey-lethal. Still more pronounced is the lack of curvature in the region of the lateral front cusp which has a very irregular shape, is wrinkled and merges irregularly into its surroundings. The cingulum is completely suppressed. Evidently this is the most abnormal region. Again, as in the lower first molars of the grey-lethal, the surface is not smooth and regular, but shows small depressions, chiefly on the lateral surface and the front parts. The irregular surface of the lateral front cusp has already been mentioned.

All these anomalies of shape suggest that the grey-lethal teeth have grown under pressure. What has happened seems to be this. At the time when the then uncalcified tooth germ gets enclosed into the socket, it has not yet reached its definite size. Now, when it grows, in a normal animal the surrounding bone responds by absorption to fit the requirements of the growing germ. Under normal conditions, therefore, shape and size of the socket is always in harmony with the dimensions of the tooth. In other words, the growth tendencies of the tooth determine size and shape of the socket.

This situation seems to be reversed in the grey-lethal. Here the bone of the socket does not yield to the growth pressure of the tooth germ. Accordingly the tooth germ has to adapt itself to the space available. It does not respond by stopping growth altogether. The growing germ, chiefly the enamel organ, is therefore bound to get deformed. In the lower first molars, the socket does not allow for a transverse expansion, but there is no obstacle to a longitudinal growth. The tooth, therefore, gets compressed in bucco-lingual direction, but compensates by longitudinal elongation. In the upper first molar, too, there are obstacles to a transverse expansion. In the present case, however, the tooth cannot compensate for this by longitudinal evasion. Under such circumstances, the only possible answer can be erection of the cusps. When calcification starts, the cusps are petrified in these positions.

The upper second molars of the grey-lethal show no anomalies of crown shape whatever. The lower second molars and the wisdom teeth are very nearly normal, the differences being almost negligible. In particular, there are no irregularities on the surface. That would suggest that their growth tendencies were practically exhausted at the time they were enclosed in the socket. Their development was therefore not hampered by too narrow walls of unyielding bone.

HISTOLOGICAL EVIDENCE

If this interpretation of the facts is correct, it should be demonstrable histologically. Suitable stages of development are those in which the tooth has already formed some enamel and dentine, but in which the tooth of the normal has not yet started erupting. For the first molars this stage is found at about the 9th or 10th day after birth. Longitudinal and transverse sections were therefore cut serially through grey-lethals and normal litter-mates of

that age. The results are shown on Pl. I. Fig. 1 represents a longitudinal section through all the upper molars of a normal. As is clearly seen, the first molar is everywhere clear of bone, there being an interval of normal width all round it. Fig. 2, a corresponding section through a grey-lethal jaw, exhibits a totally different situation. A big mass of cancellous bone presses hard against the tooth on its front part, i.e. at the expected place. The erection of the cusps which results from this is clearly seen, and it is further apparent that the effect is strongest in the neighbourhood of bone pressure. Single spicules of the spongy bone mass project towards the tooth, leaving here impressions on the surface mentioned previously in this paper. Furthermore, it is seen that the pressure, under which the tooth grew, could not be passed on to the second molar since a bony interdental septum closes the socket from behind. This offers the explanation why that tooth is not at all affected by the pressure under which its neighbour developed. In fact it develops under perfectly normal space conditions, as might have been predicted from its normal shape.

Figs. 3 and 4, Pl. I, represent transverse sections through the front part of the first upper molar, in the region of the deformed lateral front cusp. Here again, the reason for the deformity in the grey-lethal is clearly a mass of cancellous bone which in the normal course of events is removed by resorption. Figs. 5 and 6 show transverse sections through the tooth farther back. Here there is hardly any lateral pressure on the grey-lethal tooth, while a spicule hindering its median expansion is obvious.

Figs. 7 and 8 represent transverse sections through the lower first molar. The compression of the grey-lethal tooth acts mainly from the lateral side as seen by the position of projecting spongiosa. This situation corresponds exactly with Brash's conclusions about the growth of the mandible.

The same type of causation is found throughout. Where there is a deformity in any of the teeth, its cause, in the form of persistent spicules, is readily found. Histological evidence, therefore, leaves no doubt of the correctness of the interpretation that the tooth shape anomalies are due to lack of bone absorption.

THE UPPER INCISOR

A few words may be added about the upper incisors. In a normal mouse, this tooth has an outline which is almost exactly a segment of a circle. This applies already to the original tip, which is soon worn off by use. The axis of the tooth lies exactly in one plane. The corresponding tooth of the grey-lethal has an outline like an eagle's beak. The tip is strongly turned downwards so that the dorsal surface has two different curvatures meeting each other in an obtuse and rounded angle. It seems that the extreme tip immediately after the first dentine and enamel is laid down is subjected to some pressure from behind. This pressure is caused by the fact that the growing back end of the tooth finds resistance against unyielding bone. That results in an angle being

formed between the tip which is already calcified or at least stiffened by dentine and enamel, and the rest of the tooth which is still flexible. Actually, this pressure does not act exactly along the axis of the tooth. In consequence the tooth is forced into a very shallow spiral, the tip pointing somewhat towards the middle plane of the body whereas the back opening faces laterally.

The position of the bending on the tooth allows for an estimate of the time at which it occurs during development. If calcification of this tooth follows the same course as in the rat, this should have happened during the first day after birth (Addison & Appleton, 1915).

A very similar situation to that described here for the upper incisor sometimes occurs in human teeth due to an external trauma. If the calcified parts of a developing tooth are forcibly dislocated as compared with the uncalcified parts, but both continue their development, a bend with a distinct angle between the two parts may result. This condition, chiefly found in the most exposed front teeth, is called "dilaceration" in *Dental Surgery* (Bennett, 1931).

When comparing the width of the dentine and of the pulp cavity of upper incisors in older animals, one is struck by the fact that the grey-lethal has, at least in the front parts of the tooth, a considerably thicker layer of dentine, but a much narrower pulp cavity. This, however, is not yet so in young animals of about 10 days of age which do not exhibit any appreciable difference. The reason for this is very simple. In a normal tooth which is worn off by use at a regular rate, the single odontoblast has only a limited period during which it may lay down dentine. This period lasts from the time it starts functioning at the back end of the tooth until it approaches the tip where its activities are automatically stopped by the formation of secondary dentine by which the pulp cavity is obliterated. In the grey-lethal, however, the tooth is not cut and therefore not worn off. No secondary dentine is therefore formed near the tooth tip. The odontoblasts are therefore able to go on laying down dentine as long as the animal lives and their physiological condition allows. This results in a very thick layer of dentine, but a very narrow pulp cavity.

DISCUSSION

The histological investigation leaves no doubt that the explanation deduced from the shape of the grey-lethal molars is essentially correct. We have, therefore, a complete reversal of normal development. Whereas in the normal mouse or any other mammal size and shape of socket is determined by the growth of the tooth, the size and shape of the tooth in the grey-lethal is limited and influenced by that of the socket at the time it becomes enclosed in it. Teeth which have still to grow after that time are deformed, while teeth whose growth was completed then develop normally.

Actually, a similar situation was described in the grey-lethal previously but not fully understood then. The lower incisors of these animals, instead of growing in the normal way backwards till they reach the basis of the condyloid

process, find a way out of the mandible through the mental foramen. This phenomenon is essentially the same as the much less obvious anomalies described in this paper. Here again, the bone does not yield to the pressure of the backward-growing end of the tooth.

This situation is of some importance for the understanding of the bone anomaly of the grey-lethal. As mentioned above, there is no secondary bone absorption. Now it was doubtful whether this was due to a lack of stimulus for absorption or to some other reason. What the actual stimulus is which causes bone absorption, for example, at the surface of a long bone, is unknown. It was therefore doubtful whether the unknown stimulus was absent for one reason or another, or whether the stimulus acted normally, but the bone could not respond to it. Now the situation is clear. For, in the case of a socket, the stimulus for absorption is known to be the growing tooth germ. If that germ grows vigorously, but the socket does not respond to its proper stimulus, it is evident that the lack of secondary bone absorption is due to the inability of the bone to respond, not to the absence of the stimulus.

This conclusion is of some importance for the general conception of the relations of external and internal factors in bone development. It is well known that bone responds to pressure by absorption. That has been worked out for the relation of tooth and socket in great detail by Gottlieb & Orban (1931). From work of this kind it was concluded that there is a direct response of bone to pressure, a conclusion which was fully justified by the facts then known. In the light of the facts described in this paper, this relationship turns out to be more complex than then assumed. If the bone is to respond to pressure by absorption, it must be able to do so. The ability of bone to react, however, is evidently rooted in hereditary factors. If these factors act normally, as is the case in all known animals with the exception of the grey-lethal mouse, the relationship between pressure and absorption appears to be direct. If, however, this hereditary mechanism is upset, as in the grey-lethal, the stimulus by itself is unable to induce absorption in the bone. The old scheme was:

STIMULUS (Pressure) ⟶ RESPONSE (Absorption)

Now we have to fit in a new link:

STIMULUS (Pressure) ⟶ HEREDITARY BASIS FOR RESPONSE ⟶ RESPONSE (Absorption)

This link is disturbed in the grey-lethal. No response, therefore, can take place. The physiological mechanism of the hereditary basis is unknown as yet and is the subject of further studies.

In the light of these facts, certain suggestions previously made by the author need revision. It was shown that in the grey-lethal the infra-orbital foramen is considerably narrower than in a normal mouse. Incidentally, the

anterior part of the Masseter which originates in that neighbourhood is very poorly developed. As a tentative suggestion the explanation was put forward that this is due to the comparative inactivity of the muscle. Since the grey-lethal does not cut its teeth it does not nibble, and consequently uses its masticatory muscles to a much lesser extent than a normal mouse. The explanation given then amounted to the assumption that in that case the narrowness of the foramen was due to the lack of a proper stimulus for absorption. It seems now that that assumption is untenable. The causal relationship is probably reversed. Not: the hole is small since there is only a weakly working muscle which does not stimulate enlargement. But: the muscle cannot grow larger since the foramen is narrow. In this connexion it is irrelevant that the muscle is little used anyhow.

As has been shown in this paper, all the anomalies in tooth shape in the grey-lethal are secondary consequences of the lack of bone absorption, and they all develop after birth. In the hierarchy of causes, therefore, the bone anomaly is nearer the original gene action than are the tooth shape anomalies dependent on it.

SUMMARY

In normal development, size and shape of socket are determined by the growth tendencies of the tooth germ. In the grey-lethal mouse the growth tendencies of the tooth are limited by the width and size the socket had when the tooth became enclosed in it. This leads to the conclusion that bone reacts by absorption to pressure only if the hereditary basis for doing so is undisturbed. The relation between stimulus and response is therefore not direct, as assumed hitherto, but subject to conditions based in the hereditary constitution of an animal.

It is shown that all the anomalies of tooth shape in the grey-lethal are caused by the lack of secondary bone absorption which is therefore a step nearer to the original gene action.

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DESCRIPTION OF PLATE I

The technique is the same for all the preparations. Suza fixation. Haematoxylin, van Gieson; 7μ; 35 times enlarged.

The animals from which Figs. 1 and 2 were made were litter-mates, 9 days old. Figs. 3, 5 and 7, and 4, 6 and 8 respectively, were taken from another pair of animals (litter-mates) of 10 days. Despite this age difference, they represent practically the same stage of tooth development (just before the eruption of the incisors), since the older animals came from a large litter which develops more slowly.

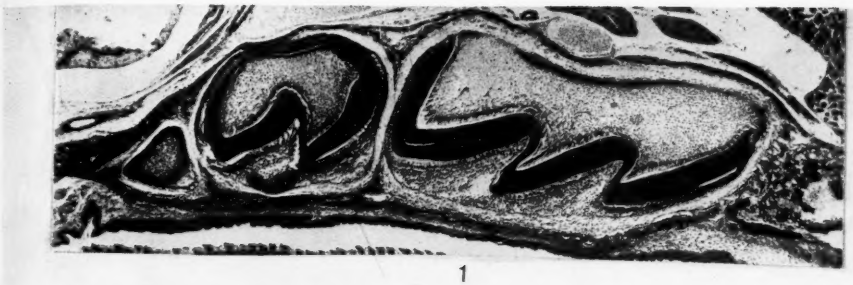
In these young stages, the enamel freshly laid down is not dissolved entirely by the process of decalcification. It is seen as a darkly staining layer in all the sections with the exception of Figs. 7 and 8, where it fades away towards the tips of the cusps. The approximate planes of the sections are indicated in Text-figs. 2 and 4.

Figs. 1 and 2. Longitudinal sections through the upper molars of normal and grey-lethal. The first molar of the normal is clear of bone all round. In the grey-lethal dense spongiosa is pressing against the front parts of the tooth, leaving marked impressions on the surface and leading to erection of the cusps. No pressure acts upon the second molar of the grey-lethal.

Figs. 3 and 4. Transverse sections through front parts of right upper first molar of normal and grey-lethal. In the grey-lethal tooth spongiosa spicules press into the tooth mainly from the lateral side where there are considerably more in the actual preparation than are brought out in the photograph. One spicule presses strongly on the median side. On the lateral side the enamel organ is complexly invaginated by spicules; such spicules are covered with a layer of adamantoblasts which have deposited enamel on the bone surface.

Figs. 5 and 6. Transverse sections through left upper first molars of normal and grey-lethal, farther backwards than the last two sections. There is little direct pressure in this region acting on the grey-lethal tooth except for one spicule pressing from the median side, strongly affecting the curvature in that neighbourhood. Note, too, the steeper shape of the middle cusp.

Figs. 7 and 8. Transverse sections through left lower first molar of normal and grey-lethal. In the abnormal tooth the resistance to growth is exclusively on the lateral side where there are many more spicules than are brought out in the photograph. They form a strong obstacle particularly for the downward growth of the lateral wall (compare Text-fig. 1).



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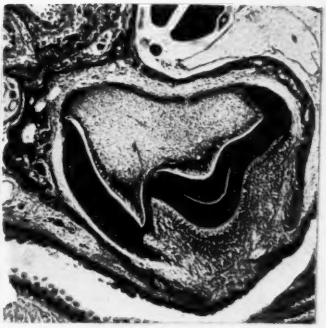

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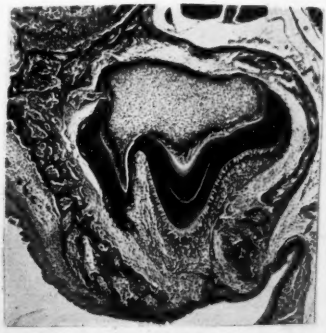

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UNIV. OF MICH.

THE OSSA SUPRASTERNALIA IN WHITES AND AMERICAN NEGROES AND THE FORM OF THE SUPERIOR BORDER OF THE MANUBRIUM STERNI

By W. MONTAGUE COBB, M.D., Ph.D.

Anatomical Laboratories of Western Reserve University and Howard University

I. INTRODUCTION

In this presentation are described the results of an investigation of the variation and incidence of suprasternal bones and the form of the superior manubrial border on human and anthropoid sterna in the collections of the Hamann Museum of Western Reserve University. For access to the material and laboratory facilities the author is deeply indebted to Prof. T. Wingate Todd.

A total of 2238 human sterna in three series were examined. The first series comprised 1010 specimens, 544 white and 466 Negro. These bones were studied from roentgenograms checked when necessary by examination of the actual specimens. The roentgenograms had been taken by Prof. Todd over a number of years as the sterna became available from the dissecting room. By special provision the sterna had been removed from the cadavera with the clavicular ligaments attached and as far as possible intact. In this way minute episternal ossicles were detected which otherwise would have been lost in maceration. The second series included 1113 sterna, 776 white and 337 Negro, examined after maceration. The third series consisted of the roentgenograms of 65 juvenile sterna, 25 white and 40 Negro. The anthropoid series was composed of the macerated manubria of 61 gorillas, 38 chimpanzees and 8 orangs.

II. REVIEW OF THE LITERATURE

In most anatomical texts mention of suprasternal bones is limited to a brief description of the occasional ossicles with a suggestive statement concerning their morphological significance (11, 15, 23, 31, 41, 49, 54, 63, 71, 73, 82, 83, 87). The older works of Henle (36), Bardeleben (7), Gegenbaur (30), Poirier and Charpy (69), and Quain (14), give more extensive discussions which may no longer be considered adequate. Martin (58) summarizes studies of the incidence and variations of the suprasternalia.

Episternal bones were first mentioned in a short paragraph by Béclard in 1820(8):

Il existe quelquefois, et j'en possède des exemples sur des sternum d'environ trente-cinq ans, deux points osseux pisiformes, placés l'un de chaque côté, sur l'échancrure trachélienne du sternum. Ces points, que l'on peut appeler présternaux ou sus-sternaux, sont peut-être le rudiment de la fourchette ou clavicule furculaire de certains animaux.

The first detailed description, however, was furnished by Breschet in 1838(12), who stated that he had earlier directed the attention of Béclard to the ossicles, so that subsequent writers have generally acknowledged Breschet as the discoverer of episternal bones. Breschet's description, remarkable for its accuracy and completeness, was illustrated by several excellent figures. He described, precisely, episternal cartilages, separate episternal ossicles and episternal bones which had fused to the manubrium, and he emphasized the uniform position of the episternal structures on the posterior portion of the superior manubrial margin. His account is quoted in part:

Pendant l'exercice de nos fonctions de chef des travaux anatomiques de la Faculté de Médecine de Paris, nous avons eu assez souvent l'occasion de rencontrer des sternum dont l'extrémité supérieure était surmontée de deux noyaux osseux ou cartilagineux. Nous en avons remis plusieurs exemples à Béclard, qui s'occupait alors d'un travail sur l'ostéose, et il en a dit quelques mots dans un supplément de son mémoire....Nous avons de nouveau observé plusieurs cas d'existence de ces pièces osseuses à la partie supérieure du sternum, et ne voyant pas dans les traités d'ostéologie d'indications suffisantes de ces noyaux osseux, nous avons pensé qu'il ne serait pas sans intérêt de les décrire et de les faire représenter....[Describes cases.] L'extrémité claviculaire de cet os [sternum] offre tout-à-fait en dehors l'insertion du cartilage de la première côte au sternum, plus en dedans deux facettes articulaires, encroûtées de cartilages, pour recevoir la clavicule, et vers la partie la plus interne de ces surfaces, deux productions cartilagineuses dirigées en haut et inclinées un peu en arrière, séparées l'une de l'autre par l'intervalle qu'on nomme la fourchette. Ces corps rudimentaires non-seulement sont plus en dedans, mais encore ils sont inclinés plus en arrière que les surfaces qui reçoivent la clavicule. Ces productions se trouvent donc tout-à-fait en arrière et en dedans de l'insertion sternale du muscle sterno-mastoidien. Sur cet os desséché, ces corps n'étant que cartilagineux, sont faiblement exprimés; mais dans l'état frais ils étaient très distincts.

Ici l'on voie sur l'extrémité cervicale ou supérieure de cet os [sternum], tout-à-fait en dehors, deux larges surfaces articulaires, concaves de dedans en dehors, lisses, destinées à recevoir la clavicule. Ces surfaces sont séparées par une crête dirigée de devant en arrière, de deux autres facettes, beaucoup moins grandes, circulaires, regardant en haut, un peu arrière et en dehors, lesquelles surfaces sont surmontées de deux noyaux osseux, arrondis sur tous les points de leur étendue, excepté sur le côté par lequel ils sont en rapport avec le sternum. Quoique placés sur l'extrémité supérieure ou claviculaire du sternum, cependant une ligne transversale qui séparerait cet os en deux moitiés égales l'une antérieure et l'autre postérieure, laisserait en arrière les deux noyaux osseux, comparables, bien que plus volumineux, aux os pisiformes du carpe. Ces deux os sont unis entre eux, vers leur côté interne, par un ligament transversal. Une membrane synoviale recouvre toute la surface par laquelle ils sont en contact avec le sternum, et ils glissent sur ce dernier os auxquels ils sont unis par de petites fibres ligamenteuses circulaires. C'est donc une véritable diarthrose temporaire....Sur ces deux os [sterna], les petites pièces osseuses dont nous

faisons l'histoire, bien qu'unies solidement au sternum, permettent de voir bien nettement la ligne de séparation du sternum avec les os sus-sternaux. Sur toutes les pièces que nous avons observées, comme sur toutes celles que nous avons fait représenter, on reconnaissait que les os sus-sternaux n'étaient pas regulièrement arrondis, car ils sont un peu allongés transversalement et aplatis sur le point correspondant du sternum.

In 1840 King (43), with a brief note, presented a sketch of a pair of episternal bones which came under his observation. In 1843 Knox (45) gave the second detailed description of episternal bones, from a case encountered in his dissecting room, and completely confirmed the findings of Breschet.

The specimen I examined with a good deal of attention, but found little to add to the extremely accurate descriptions of preceding observers. Situated behind the sternal attachments of the sterno-mastoid muscles, and mesially in respect to the articular surface for the clavicles, the presternal bones are attached by their bases to the inner or deeper margin of the notch of the manubrium of the sternum; they are of a pyramidal form, and approach each other slightly at their summits. The base of each appeared to me encrusted with cartilage, and there existed a close but distinct movable joint, with a synovial apparatus, and strong ligamentous bands of a peculiar reddish colour, between them and the sternum; one was less movable than the other and a ligamentous band connected them to each other. A few muscular looking fibres, but extremely short, ran from the sternum to these bones.

After Knox the authenticity of Breschet's original descriptions seemed established and subsequent workers became concerned with the homology, embryology and incidence of the episternal structures. Additional careful descriptions of the ossicles and their anatomical relationships and attachments have been furnished by Luschka (52, 53), Carwardine (17), Anthony (4), Lickley (50), Stein (78) and Dixon (22). Following Breschet, dissection of suprasternal cartilages was reported by Luschka (53), Ruge (75), Carwardine (17), Mackay (55), Paterson (66), von Eggeling (25) and Lickley (50). Variations of the ossicles observed on recent and dry material have been described by Luschka (52), Strauch (80), Carwardine (17), Bogusat (9), Paterson (67), von Eggeling (26) and Malaguzzi-Valeri (56). A long series of comparative and embryological investigations seems finally to have shown that the suprasternalia are rudiments of the epicoracoids of the primitive shoulder girdle, other interpretations having considered them respectively; rudiments of the furcular clavicle of Birds (8); the ventral ends of cervical ribs (12, 75, 2, 10); the interclavicle or episternum of lower Vertebrates (28, 26, 10); cartilage separated from the end of the clavicle (30, 34, 37, 47); a clavicular epiphysis (20); a sternal epiphysis resulting from accessory centres of ossification (72, 54); sesamoids (4); and remnants of the precoracoid (17, 50). Only the studies of Strauch (80), Bogusat (9), Paterson (67), von Eggeling (24) and Barchielli (5), have been concerned with the incidence of episternal bones. Lossen and Hofer (51) demonstrated the ossicles in the living in a roentgenogram of a male of 50 years. The monograph of von Eggeling (25) is the most comprehensive treatise on the subject.

Descriptions

Luschka described three cases of episternal bones. Their general form resembled a pisiform with a free convex upper surface and a flattened under surface for articulation with the sternum. One specimen, however, presented four distinct surfaces. The largest ossicle observed had a height of 8 mm. and a breadth of 12 mm. The bones were regularly situated on the hinder portion of the superior manubrial margin adjacent to the clavicular notches. On each manubrium somewhat posteriorly projecting bony elevations with flat or slightly convex free surfaces served as pedestals for the articulation of the suprasternal bones. The ossicles were composed of cancellous tissue surrounded by a thin layer of compactum. They were invested with a relatively thick firmly adherent fibrous periosteum which filled the space between a pair of ossicles. Articulation with the manubrium was by diarthrosis or synchondrosis, the latter being more frequent. Luschka described two ligaments as being peculiar to the suprasternal bones: an anterior ligament which passed from the ventral edge of the semilunar notch obliquely upward and backward to the upper limit of the anterior surface of the suprasternal bone, and a shorter, narrower and vertically directed posterior ligament which joined the dorsal surface of the ossicle to the manubrium (cf. Carwardine). In addition the ossicles were found to be united with the interarticular disc of the sternoclavicular joint by a strong ligamentous mass. Luschka particularly emphasized that neither the interclavicular ligament, the anterior and posterior sternoclavicular ligaments, nor the sternomastoid muscles had any special connection or association with the suprasternal bones.

Eine ganz besondere Berücksichtigung verdient das Verhältnis der Nachbarteile zu den Suprasternalknochen. Hier ist vor allem der Zwischengelenksknorpel des Sternoclaviculargelenkes, welcher eine nahe Beziehung zu jenen Knochen zeigt, indem er durch eine sehr feste Bandmasse mit dem äusseren Umfange derselben in Verbindung steht, resp. an sie befestigt ist.—Das Lig. interclaviculare steht in keinerlei Beziehung zu jenen Knochen, indem es, durch ein straffes Bindegewebe von ihnen geschieden, über sie hinweggeht. Auch das vordere und das hintere Verstärkungsband des Sternoclaviculargelenkes haben nichts mit ihnen zu schaffen, da sie nach aussen vor denselben sich ausbreiten. Ebenso findet sich, dass die Mm. sternocleidomastoidei nicht die entfernteste Beziehung zu den Ossa suprasternalia haben, indem dieselben mindestens 6 mm. nach vorn von ihnen, unter dem vorderen Rande des oberen Brustbeinausschnittes ihre Insertionen finden.

Later, elaborating, Luschka stated that the rather weak fibrous band linking the meniscus to the medial side of the clavicular incisure is notably thickened in the presence of suprasternal bones, and that beneath the interclavicular ligament are found ligamentous bands which bind the episternal bones to each other.

Both Breschet and Knox had mentioned ligamentous connexions of episternal bones to each other without reference to the normal variable interclavicular ligament. Bardeleben (7) later made a study of the interclavicular ligament on preparations from human embryos, foetuses, children and

adolescents. He concluded that the interclavicular ligament of the growing child splits in a characteristic manner. A superficial fibrous band connects the clavicles and a deeper layer runs partly between the menisci but especially between the menisci and the upper manubrial border. He termed the former the Lig. interclaviculare and the latter deeper layer the Lig. episternale. Bardeleben thus recognized the same elements described by Luschka.

Carwardine further elaborated the account. He described a case showing a well-marked T-shaped interclavicular ligament, beneath the horizontal limbs of which two ligamentous bands passed, "from behind the clavicles in a direction forwards, inwards, and downwards to the sternum, where they are attached on either side of the suprasternal notch". These bands he called "suprasternal ligaments". In this case the ligament on the right side was occupied by a suprasternal bone which was freely movable upon an articular facet on the sternum. The joint had a synovial membrane and an articular capsule formed by the fibres which bound the ossicle to the sternum. The corresponding ligament on the left side contained no bone but a slight cartilaginous thickening in its lower part.

In a second specimen Carwardine found the suprasternal ligament on the left side, "passing into an irregular scale-like nodule of bone which [showed] undoubted traces of secondary connexion with the sternum by ankylosis. This suprasternal nodule is in fact a suprasternal bone, which, though at an early period independent, has fused with the sternum at a later stage. On the right side the ligament [passed] into a tubercle representing a similar, but more advanced condition, and which, in order to adopt uniform nomenclature may be called the suprasternal tubercle." From this and one additional case showing suprasternal ligaments but no ossicles or tubercles, Carwardine concluded that suprasternal bones occurred as ossifications in the "suprasternal ligaments", which are almost constantly present in the adult; that the suprasternal bones may fuse early with the sternum and be represented by tubercles with the suprasternal ligaments attached to them; and that in other cases the episternal bones may be incorporated in the manubrium as separate centres of ossification leaving the suprasternal ligaments attached to the sites of incorporation. Carwardine regarded the occurrence of separate ossicles as a sign of incomplete ossification and development of the sternum.

Anthony noted, nearly always, the suprasternal ligaments described by Carwardine and sometimes the accompanying suprasternal tubercles mentioned by the latter:

> Voici, d'après une de nos observations, la description de cette anomalie; c'était chez un homme de 41 ans (un seul cas sur 66 dissections). La fourchette sternale présentait à ses deux extrémités deux petits noyaux osseux de la grosseur d'un pois à peu près et ayant la forme d'une pyramide triangulaire. Ils présentaient quatre faces: une inférieure, une externe, une antérieure et une postérieure. La face inférieure s'articulait avec le sternum; la face externe avec le cartilage inter-sterno-claviculaire; ces articulations étaient des diarthroses (dans un cas de Luschka l'os suprasternal se reliait au sternum par synchondrose). La petite cavité de l'articu-

lation sterno-suprasternale communiquait en avant seulement avec l'articulation sterno-meniscoidale; la cavité de l'articulation suprasterno-meniscoidale communiquait en arrière seulement avec celle de l'articulation sterno-meniscoidale; la face antérieure était recouverte par quelques ligaments longitudinaux partant du ligament interclaviculaire pour se rendre au sternum. Ces ligaments sont ceux sur lesquels s'étend Carwardine. En outre de cela quelques fibres parallèles à celles du ligament interclaviculaire reliaient l'os suprasternal à la clavicule. La face postérieure était en connexion avec le sternum et la clavicule par l'intermédiaire de fibres irregulièrement disposées mais suivant, pour la plupart, la direction du ligament interclaviculaire. Des quatres angles deux seulement présentaient des particularités dignes d'intérêt: l'angle supérieure qui se reliait au ménisque interclaviculaire et à la clavicule par des fibres parallèles au ligament interclaviculaire, et l'angle interne qui donnait attache à des fibres dont la direction était la même que celle des précédentes; de ces dernières fibres les unes s'attachaient au sternum, les autres se perdaient dans l'épaisseur du ligament interclaviculaire. Chose importante à noter, c'est que l'os suprasternal n'affectait que de simples rapports de contiguité avec le ménisque interarticulaire.

This case presented also an articulation in the middle of the xiphisternum and a presternal (sternalis) muscle.

The subsequent careful description of the relationships of episternal bones by Lickley gave essential confirmation to ligamentous arrangements reported by Luschka, Bardeleben, Carwardine and Anthony. Because Lickley's is perhaps the most photographic of the accounts it is presented *in toto* to afford comparison with the more diagrammatic descriptions of other writers:

With the upper margin of the presternum, between the clavicular articular surfaces, two bones, about the size of large pisiform bones, articulated. The articular surfaces for these bones occupied the greater part of the interclavicular notch, only a small median notch being non-articular. Each articular surface was oval in outline and nearly flat both from side to side and from before backwards.

The bones were firmly held in position by ligaments. The interclavicular ligament occupied its usual position between the sternal ends of the clavicles. From its deep surface a fibrous sheet passed down to the anterior surface of the presternum. This ligamentous sheet was specially strengthened over the anterior surface of each suprasternal bone, the whole ligament presenting therefore the appearance of two strong lateral bands with a thinner intervening portion. A thinner band passed down from the interclavicular ligament behind the bones to be attached to the posterior surface of the presternum. Traced outwards these ligaments were found to become associated with the capsule of the sternoclavicular articulation and with the anterior and posterior sternoclavicular ligaments.

The bones themselves were bound to one another by strong interosseous fibres extending between their mesial surfaces across the middle line. In addition each bone was attached to the corresponding fibroplate of the sternoclavicular joint by means of ligamentous bands passing downwards and outwards.

The presence of two cartilaginous nodules corresponding in position with these bones was noted in another case also. The sternum was taken from a girl of 18 whose general development was very defective. At the sides of the interclavicular notch were two distinct cartilaginous nodules—that on the right being the larger, each articulating with the upper margin of the presternum, and connected to it by a capsule of fibrous tissue.

Stein described a case in which the outer surface of each episternal bone was united to the interarticular disc of the sternoclavicular joint by a fibrous mass.

Dixon reported two cases, one in an adult man, the other in an adult woman. His roentgenograms showed that the architecture of the suprasternal bones resembled that of the sternum. In the male the ossicles were of conical form, large and almost symmetrically placed; those of the female were somewhat pea-shaped and placed close against the median plane. The ossicles of both male and female articulated with the sternum by diarthrodial joints. In the female there was also a diarthrodial joint in the median plane where the right and left ossicles were in contact. The capsular ligaments of the bones in the male were loose enough to permit considerable movement at the articulations. Each suprasternal ossicle of the female was "tied down to the sternum by a well-defined fibrous capsule which is specially thickened on its anterior aspect to form a rounded cord-like ligament. The anterior surfaces of the ossicles were connected by a fibrous band, the upper margin of which was continuous with the interclavicular ligament. Laterally each was connected to the clavicle by a fibrous cord, the lower and lateral edge of which was continuous with the sternoclavicular ligament." Dixon emphasized that "the suprasternal bones are not directly connected with the fibro-cartilaginous disc, and that the joints which they form with the sternum are distinct and isolated from the clavicular articulations".

In considering Dixon's failure to find the special fibrous or ligamentous connexion between meniscus of the sternoclavicular joint and suprasternal bone or cartilage reported by Luschka, Anthony, Lickley, von Eggeling and Stein, it is to be remembered that the ossicles are really embedded in a fibro-ligamentous mass of which the structural elements may be variously determined by different dissectors.

Episternal bones are preformed in cartilage. Episternal cartilages were first described by Breschet from specimens in an adult. A pair were found by Luschka in an 11-year-old boy. They were of hyaline material, with a breadth of 5 mm. and a height of 4 mm. The attachment to the sternoclavicular meniscus was clearly marked. Ruge found episternal cartilages in a male infant of 6 months, illustrated by his fig. 22. They exhibit the same form and positional relationships as the adult ossicles.

In 290 foetal sterna between 3 and 9 months, Paterson (66) found two examples of suprasternal cartilages. Upon the upper border of one female sternum of 6 months two ovoid cartilages were present, fused to each other but separate from the manubrium. On a second female sternum of 9 months two cartilages occurred, separate from each other but fused with the upper manubrial border. Mackay (55) demonstrated cartilaginous suprasternal nodules in three cases of foetal sterna of the seventh month. The cases of Carwardine and Lickley have been mentioned. von Eggeling (25) described three cases of suprasternal cartilages. In a girl of $3\frac{1}{2}$ years small hyaline suprasternal cartilages were found which did not extend to the clavicular articular surfaces. They were bound by fibrous bands to a cartilaginous manubrium. A boy of $4\frac{1}{2}$ years presented asymmetrical hyaline cartilages which were contiguous laterally

with the clavicular incisurae. The ossification of the manubrium was almost complete. A right unilateral cartilage occurred in a boy of 12½ years which did not reach the clavicular notch. Sections of the cartilage revealed a centre of ossification. This is the earliest age at which a calcified nodule has been reported. In a study of the ossification of the sternum embracing 450 specimens of which 31 were foetal, 68 newborn, and the remainder ranging up to 16 years, Markowski did not report a single case of suprasternal cartilages.

The most remarkable of the variations of suprasternal bones is their occurrence as nodules of bone fused to the superior manubrial border which alter the character of the jugular notch. This relationship between the contour of the upper margin of the manubrium and suprasternal bones was first perceived by Luschka (53). He stated that in addition to normal variation as to size and form, the semilunar notch manifested a great variability because of the existence of suprasternal bones. While the most common form of jugular notch presented a concave rounded margin which was steep behind and sloped forward gradually, there would frequently be found on the highest posterior part of this margin, on either side, inconspicuous rounded rough eminences which according to the breadth of the notch either directly adjoined the clavicular incisurae or were separated from them by a deeper intermediate area. Often, especially on the narrower semilunar notches, there occurred in the position of these inconspicuous elevations discrete projecting bony processes which sprang up between the semilunar notch and the highest point of the clavicular notch. These processes or tubercles were attached to the sternoclavicular menisci by short bands of fibres and were held by Luschka to be undoubtedly manifestations of suprasternal bones. An interpretation was later made independently by Carwardine as has been seen.

Die Grösse und die Gestaltung der Incisura semilunaris zeigt schon innerhalb des Breitegrades der Normalität bedeutende Schwankungen und besitzt bei der Existenz von Suprasternalknochen einen sehr abweichenden Typus. Bei ganz regelmässiger Bildung stellt sie einen konkaven abgerundeten Rand dar, welcher gegen die hintere Seite steil, nach vorn dagegen ganz allmählich abfällt.—Gegen sein Ende geht der erhabenste Teil dieses Randes jederseits sehr häufig in eine rundlich Rauhigkeit über, die jedoch ihrer Flachheit wegen die Aufmerksamkeit kaum auf sich zieht und, je nach der Breite der Incisura, entweder mittelbar an den Schlüsselbeinausschnitt angrenzt oder durch einen schmalen, etwas vertieften Zwischenraum von ihm geschieden ist.—Nicht finden sich, zumal bei schmaler Incisura, an den Stellen dieser unscheinbaren Erhebungen grössere, durch ihre Höhe und durch ihren Umfang sehr augenfällige, rundliche Knochenvorsprünge, welche sich hügelartig zwischen der Incisura semilunaris und dem höchsten Punkte des Schlüsselbeinausschnittes erheben.—Diese kleinen oder grösseren Knochenerhebungen nehmen die Aufmerksamkeit dadurch in Anspruch, dass an ihnen durch einen kurzen Bandstreifen die Anheftung des Meniscus der Sternoclavicularverbindung statt hat und dass sie ohne allen Zweifel die Andeutungen der unter Umständen als einige Skeletteile auftretenden Suprasternalknochen sind.

In the first statistical study Strauch recognized in his classification separate episternal bones and episternal tubercles. Bogusat found in his series three cases in which episternal bones occurred on one side only. Paterson presented

an excellent plate of ten specimens showing: separate ossicles; a pair of ossicles, one of which was fused and the other separate; suprasternal tubercles; tubercles with articular facets; the suprasternal border raised into a median projection; and normal semilunar incisurae, one broad and shallow, another narrow and deep. He mentioned that the ossicles might be fused together and be bilateral or median in position. Von Eggeling stated that between sterna with fully formed separate ossa suprasternalia and those with no trace of the ossicles there exists a large series of intermediate stages which demonstrates the gradual disappearance of this once prominent skeletal feature. He illustrated with a plate similar to Paterson's but showing some slightly different stages. Malaguzzi-Valeri described with illustrations of roentgenograms two cases of episternal bones, one with a unilateral ossicle, the other with symmetrical bones, one of which was fused and the other free.

Homology

The determination of the phyletic significance of suprasternal bones was a difficult problem because its solution was dependent upon the unravelling of certain of the perplexing homologies of shoulder girdle, sternum and ribs. The approach was through comparative and embryological investigations, both being pursued concurrently by several workers.

Béclard's original suggestion that episternal bones were related to the avian furcular clavicle received no further support. Breschet, and afterwards Bonnet (10), considered them the ventral rudiments of seventh cervical ribs. Ruge (75) also held probable an origin from a seventh cervical rib, although he made no definite commitment. Luschka (53), however, whose study apparently escaped the attention of Ruge, reported a case in which well-developed episternal bones were present on a manubrium which had been joined by a seventh cervical rib below the clavicular articular facet. Leboucq (47), Ledouble (48), Stein (78) and von Eggeling (26) confirmed this finding with similar cases. Turner (88), Albrecht (2) and Keith and Herkelet (42) also showed that the ventral attachment of a seventh cervical rib was always below the clavicular facets.

Malaguzzi-Valeri (56) agreed that this evidence excluded origin from a seventh cervical rib but suggested that derivation from a higher cervical rib, probably the sixth, was possible because episternal bones are preformed in cartilage and are generally accompanied by cervical ribs and because, like the ventral ends of the latter, they tend to be asymmetrically developed and become incorporated in the manibrium. No positive evidence has rendered this theory tenable. Episternal bones, therefore, could not be considered the rudiments of ventral ends of cervical ribs. They must represent elements of either the shoulder girdle or the sternum.

The sternum itself, however, is now held to be a shoulder girdle derivative, as shown by the ontogenetic and comparative studies of Paterson (67), Kravetz (46), Whitehill and Waddell (90), Hanson (35) and Gladstone and

Wakeley (32). It is formed from the sternal bands and a median anlage cranial to the latter, all of which arise independently of the ribs. As the sternal bands fuse the median anlage is incorporated with them. The suprasternal elements, it now appears, are variable components developed from two additional lateral condensations of mesoderm between the median anlage and the inner ends of the clavicles. The median anlage may be recognized in embryos of from 16 to 22 mm., but not earlier (32).

The anlagen of human suprasternal bones were first described and illustrated by Ruge as two nuclei found in embryos of 24, 25, 30 and 30 mm., cranial to the sternal bands and between the sternal ends of the clavicles. These nuclei were subsequently studied by Müller (60), Malaguzzi-Valeri (56), and Gladstone and Wakeley. Malaguzzi-Valeri contended that the nuclei were not the anlagen of suprasternal cartilages, submitting that the nuclei were constant and the ossicles rare; that the nuclei were never distinctly cartilaginous, while the ossicles were always so preformed; and that the nuclei appeared more closely linked by their structure to the clavicles than to the sternum, but the suprasternalia exhibited affinity to the sternum, often fusing with it.

The other two authors agreed with Ruge in considering the episternal nuclei the anlagen of the adult ossicles. It was the opinion of Müller and of Eggeling, later concurred in by Bardeen (6), that the paired nuclei usually fused with the sternum but might become ossified either separately or as bony projections from the upper margin of the manubrium.

Although Ruge advocated as a result of his investigation his long-accepted theory of a costal origin for the sternum, he was careful to emphasize that the significance of the "episternal" nuclei was not determined, but as previously mentioned he was inclined to regard them as ventral rudiments of cervical ribs.

Gross specimens and dissections have established the constant position of suprasternal bones on the posterior manubrial margin as inclusions in the ligamentous investment of the sternoclavicular joint often especially attached to the interarticular disc of the latter, and have demonstrated that the ossicles are not the ventral rudiments of seventh cervical ribs. Embryological studies have shown that the ossicles develop from an anlage associated with the shoulder girdle. More precise determination of the homology of the ossa suprasternalia must be sought in comparative investigations.

These comprise a large literature which cannot be said to settle the question absolutely, although the evidence clearly indicates a derivation from the epicoracoids or their cranial prolongations, the omosterna. The preglenoid portion of the pectoral girdle is considered to consist primitively of a coracoid bar which by fenestration becomes subdivided into cephalic procoracoid and caudal coracoid bars, joined ventrally by an epicoracoid (39). This condition is illustrated by the snapping turtle, *Chelydra serpentina*. Continuous caudally with the median epicoracoids is the rib-bearing sternum proper, the essential paired nature of which is evident from its formation through the sternal bands.

Cranially from the epicoracoids there may extend cartilaginous prolongations, the omosterna, which, like the costal sternum below, may become fused into one. A well-developed omosternum may be seen in the bull frog, ***Rana catesbiana***. Secondarily, the clavicles and the episternum or interclavicle appear as dermal elements. Both are present in the monotreme, ***Ornithorhyncus anatinus***. In the duck-bill the prosternum or manubrium articulates with one and a half ribs. Attached cranially to the prosternum, and hence precostal in position, are the primitive elements of the shoulder girdle. The coracoids are firmly joined to the lateral angles of the prosternum. Above the coracoids are large epicoracoids which overlap each other. In the position of the procoracoids are long clavicles which reach from the acromial processes to the mid-line, where they meet. Co-extensive with the clavicles is the horizontal portion of a large T-shaped interclavicle, the broad vertical bar of which overlies the epicoracoids and rests upon the prosternum. The interclavicle is often described as supporting the clavicles. It is thus apparent that in their primitive positions the clavicles are separated from the costal sternum by a wide interval, represented in the cartilaginous girdle by the epicoracoids which extend forward to the procoracoids. Since the membranous clavicle invested or at least replaced the procoracoid, the same distance is spanned by the vertical portion of the membranous interclavicle. In the higher Mammals, however, the clavicles have come to articulate directly with the sternum just cephalic to the attachment of the first rib. The fate of the primitive elements intervening between clavicles and costal sternum, namely, procoracoid, epicoracoid and interclavicle, is the problem which long intrigued morphologists.

Between the primitive condition in the monotreme and the advanced arrangement in Man are certain intermediate conformations in which the clavicles do not directly articulate with the sternum. These are found in Marsupials, Edentates, Insectivores, Rodents and lower Primates, animals which are either primitive, as the Marsupials, or have extensive use for the upper extremity in burrowing or brachiating. Examples of most of these forms are figured in Parker's comprehensive monograph on the shoulder girdle.

The elements which intervene between clavicles and sternum of the monodelphia mentioned are the T-shaped Marsupial episternum and the praeclavia of Rodents and Insectivores. As both of these elements are preformed in cartilage, the membranous interclavicle may be dismissed at once as a possible precursor. Although Nauck (61) has recently claimed that the interclavicle of Monotremes is in reality, like the clavicle, a mixed bone formed through fusion of the dermal interclavicle and part of the primordial cartilaginous prosternum, the monotrematous interclavicle has both topographically and functionally the same relationships as that of the saurian. The interclavicle is in relation with the anterior or ventral surface of the costal prosternum, while the praeclavia and suprasternalia are in relation with its posterior or dorsal surface. Parker held that the saurian interclavicle was not represented in Mammals

above the Monotremes. Broom (13) described the loss of the interclavicle as a change to give the clavicles greater mobility, as the median detachment of the coracoids afforded greater freedom of movement to the limbs.

The possible precursors of the ossa suprasternalia are thus reduced to medial procoracoid, cranial epicoracoid or its adjacent forward prolongation, the omosternum.

In the opossum, *Didelphys marsupialis*, Gegenbaur found a small T-shaped cartilaginous structure which he called the "episternum". The clavicles articulated with the lateral bars of the T. These portions Gegenbaur homologized with the praeclavia of Rodents and the human interarticular disc. The middle piece of the T he considered represented by human episternal bones. Parker directed attention to a distinct fibrous tract which separated the middle piece of the T interpreted by him as prosternum from the arms (omosternum). The homology of this middle piece is uncertain. Götte described in the embryo mole a cartilaginous T-shaped episternum formed of paired median bars which lay ventral to the manubrium and extended forward to articulate with two lateral bars for the clavicles. This cartilaginous structure in the position of the membranous interclavicle was held by Gladstone and Wakeley to represent in the lateral bars, the articular cartilages between the omosterna (praeclavia) of the mouse and the interarticular disc in Man, and in the median paired elements, the omosterna. How the latter authors explain the position of Götte's median bars as omosterna *ventral* to the manubrium is not clear.

Among the Edentates Luschka found in the six-banded armadillo, *Dasypus sexcinctus*, a small bone on each side through which the clavicles articulated with the sternum. In the nine-banded armadillo, *Dasypus novemcinctus*, he found a single bifid skeletal element at the cephalic end of the sternum, the prongs of which furnished attachment for the clavicles. The latter condition Luschka thought anticipated the former, and he considered the praeclavia of *Dasypus sexcinctus* the homologues of suprasternal bones.

The praeclavia of Rodents and Insectivores present great variability but all are in relation with the posterior aspect of the superior portion of the manubrium (prosternum), and some pairs overlap in the mid-line.

The investigation of Oehngren (62), who studied foetuses of several species of Insectivore, is perhaps most illuminating. In these animals the episternalia or praeclavia connected the clavicles with the sternum. The episternal structures occupied the dorsal position just described in relation to the manubrium and were considered by Oehngren the homologues of the human suprasternalia. This author pointed out that the overlapping of the episternal elements in *Microgale dobsoni* was directly comparable to the overlapping of the epicoracoids in certain Amphibia (*Anura arcifera*), in which the epicoracoids occupied a similar position in relation to the sternum.

It may be accepted, therefore, that the praeclavia are homologues of the epicoracoids, and there remains to be established only the relationship between the praeclavia and suprasternal bones. Luschka, Eggeling and others have

considered with Oehngren that the suprasternal bones and praeclavia are homologous. This phase will be treated further in the discussion below.

Among the Primates, Burmeister's illustration of a skeleton of *Tarsius* shows well-developed episternal ossicles (16). There is no comment in the text. Parker gives figures of the praeclavia of *Mycetes seniculus* and *ursinus*. The suprasternalia are known to occur in *Cercocebus aethiops* and *Pithecus satyrus* (25).

Due apparently to misunderstanding of the nature of an epiphysis, Dawson (20) recently suggested that the praeclavium of the rat, *Mus norvegicus albinus*, was homologous with the sternal epiphysis of the clavicle instead of the epicoracoids. Parker had previously represented the praeclavium of *Mus musculus* as the omosternum which itself he derived from the epicoracoid.

A clavicular origin for the suprasternalia had been postulated before, however, for Gegenbaur (30), Götte (34), and Hoffmann (37) construed them to be derived from cartilage separated from the end of the clavicle. But even if this were true the clavicular cartilage according to present concepts would have to represent a procoracoid source.

Brief mention may be made of certain other suggestions on the derivation of episternal bones. Rambaud and Renault (72) stated that they had never found the suprasternal bones described by Breschet. They described and illustrated separate sternal epiphyses for the clavicular articular facets and suggested that these plaques might be the suprasternal elements despite the obvious totally different form and position of the two. Secondary centres of ossification for the clavicular facets are mentioned also by Radasch (71). Curiously enough, Macalister (54) wrote, "Thin crusts of epiphyses appear on the clavicular surfaces about the age of twenty-five and soon become united", but his illustration of these crusts plainly showed well-developed suprasternal bones.

Later Rambaud and Renault described as sesamoids two ossicles which were true suprasternal bones. They held that these sesamoids were the suprasternalia.

Anthony at first regarded the suprasternal bones as rudiments of the epiprecoracoids. He later changed this view and interpreted them, "comme des sésamoides d'apparition récente chez un animal où les mouvements de la clavicule sur le sternum ont acquis leur maximum de liberté et où, par le fait de la disposition même des parties, les frottements atteignent une grande intensité". The sesamoid theory is hardly to be entertained seriously, however, for the ossicles do not occur in the tendon of any muscle, and their location is not the site of particular functional stress. Neither do they by their presence appear to strengthen, protect or functionally improve the sternoclavicular joint.

The evidently erroneous concept that suprasternal bones might be represented by accessory centres of ossification in the manubrium beneath the sites attachment of the suprasternal ligaments was introduced by Carwardine. His Fig. 4 shows two such centres above the usual single large centre for the manu-

brium. Unlike the accompanying diagrams, however, this figure did not illustrate an actual case. It was a hypothetical diagram based on the account of Breschet. Examination of Breschet's original article does not reveal justification for it. This author merely admits that the sternum may be formed from two series of laterally placed nuclei.[1] After his extensive investigation of the subject Paterson concluded that centres of ossification in the sternum were of no essential morphological importance. The centres hypothetically introduced by Carwardine were not found in the series of young sterna studied by Anthony, Mayet (59) or Markowski, and in so far as we have been able to determine they have never been found to exist.

Incidence

Only the investigations of Strauch (80), Paterson (67), Bogusat (9), von Eggeling (24) and Barchielli (5) have been concerned with the incidence of episternal bones. Of these the latter three also gave attention to the form of the superior border of the manubrium.

In 200 sterna at Dorpat, Strauch found 4 cases (2 per cent.) with distinct episternal bones on both sides and 1 case (0·5 per cent.) with one ossicle. He noted only 6 cases (3 per cent.) with suprasternal tubercles.

Out of 563 sterna of various ages in Liverpool, Paterson found only 1 case bearing a pair of separate suprasternal ossicles. In another case he noted a pronounced tubercle on one side and a sessile prominence on the other, surmounted by an articular facet. In 45 cases (8 per cent.) there were found on the suprasternal border two lateral projections in the form of ridges or tubercles, sometimes provided with articular facets. In 51 cases (9 per cent.) there was a tendency toward the formation of a single median protuberance in place of the notch. The remaining 467 sterna were classed as possessing a normal suprasternal notch.

Bogusat, at Königsberg, found 3 unilateral episternal bones on 120 sterna (2·5 per cent.). Episternal tubercles were not mentioned.

Von Eggeling classified his Strassburg material in a manner similar to that of Paterson. In a series of 226 sterna, from which 5 were eliminated for pathological reasons, von Eggeling found 3 cases (1·35 per cent.) with suprasternal bones on both sides. Six cases (2·7 per cent.) had a separate ossicle on one side. Twenty-one specimens (9·2 per cent.) showed suprasternal tubercles. Fifty-three sterna (24 per cent.) had a flat or projecting upper border and 144 (65 per cent.) presented the normal jugular notch.

Von Eggeling divided his sterna into four ill-defined and overlapping groups for consideration of the superior manubrial margin. His first group was characterized by a jugular notch, of which he illustrated deep, shallow and

[1] Breschet, p. 101: "Les petites pièces osseuses, que nous signalons ici, peuvent-elles être données comme une preuve du mode de développement du sternum, par deux noyaux latéraux, et confirmer la loi de symétrie de l'ostéogénie, proposée par M. Serres?" P. 103: "...nous admettions la formation du sternum par deux séries de noyaux osseux latéraux."

narrow varieties. The second group was marked by a jugular crest, or practically horizontal upper manubrial border. The third group was distinguished by jugular tubercles. These were low rather poorly delimited eminences of which two types were described. In one the tuber was confluent with the margin of the clavicular notch; in the other it was separated from the latter by a furrow. Von Eggeling seems to have regarded the tubera as rudiments of the suprasternalia. The fourth group had ossa or tubercula suprasternalia. Von Eggeling stated that Markowski's figures showed that all these types were preformed in cartilage. The writer, however, was unable to find in Markowski's plates, which showed 180 young specimens, a cartilaginous border which had a tuber jugulare separated from the clavicular notch. The significance of this will be discussed below.

Von Eggeling directed attention to a horizontal line often distinctly visible on the posterior manubrial surface which extended between indentures in the clavicular facets and demarcated the precostal from the costal portion of the manubrium.

In 162 adult sterna Barchielli(5) found no cases of separate episternal ossicles, 3 cases (1·7 per cent.) of episternal tubercles, 3 cases (1·7 per cent.) in which the superior manubrial margin was prolonged to a point, 4 specimens (2·5 per cent.) in which this border was convex, 43 specimens (26·5 per cent.) which exhibited a straight border, and 109 (67·2 per cent.) showed the normal incisura jugularis. This author affirmed that the ossa and tubercula suprasternalia represented the ossified suprasternal cartilages free and fused with the sternum respectively, and that the various forms of convex and straight upper manubrial border represented the episternal elements fused to each other and with the sternum but incorporated in the latter to different degrees.

Thus, our knowledge of the incidence of episternal bones is limited to the results of studies of five small series of sterna of different European nationalities comprising a total of 1270 specimens. The illustrations and descriptions of the several investigators have provided an illuminating but incomplete picture of the range of variability of the ossicles.

Von Eggeling alone has presented a table showing the age and sex distribution of his material, but the series and total number of ossicles (9) and tubercles (15) found is too small to give reliable estimates of incidence.

The statement appears in Wilson's *Anatomy*(33) that suprasternal bones appear in about the thirty-eighth year. Hutchinson(40) states that they occur only at rather advanced periods of life. Von Eggeling's previously mentioned find of a centre of ossification in a suprasternal cartilage of a boy of 12½ years is the earliest osseous appearance reported. He found suprasternal tubercles in a male and a female subject between the fifteenth and twentieth years.

The racial incidence of the suprasternalia has not been investigated. Because his Strassburg material showed a much greater incidence than Paterson's Liverpool series, von Eggeling suggested that it was highly probable that different anthropological groups would show varying percentages of the ossicles.

Carwardine and Lickley associated the occurrence of episternal bones with defective osseous development in the sternum or elsewhere. Leboucq and Malaguzzi-Valeri emphasized their occurrence in association with cervical rib, Leboucq interpreting the phenomenon to be a separation of skeletal elements normally fused together.

There are numerous additional scattered comments on the form of the upper manubrium. Portal (70) stated that with a highly hafted shoulder there is usually a low clavicular notch probably due to pressure of the clavicle on the sternum. Dursy mentioned that occasionally the clavicular notches are approximated at the expense of the jugular notch. Aeby (1) observed that the superior manubrial margin may be raised up into a prominent oblique eminence. Pansch (64) noted that the lateral end of the jugular notch frequently presented elevations of varying form and size, some of which resembled fused suprasternal bones. His Fig. 2 shows such a case in which the meniscus of the sternoclavicular joint was attached to the lateral side of the process. Poirier remarked upon the varying breadth and depth of the jugular notch and stated that occasionally it terminated in a roughened region corresponding to the attachment of the superior sternoclavicular ligament. Von Eggeling is particular to point out that the functional use of the upper extremity is the essential factor determining the structure of the shoulder girdle, a fact which must be kept in mind in considering the form of the manubrium. Kirchner gave the dimensions and incidence of manubrial types in 27 sterna studied at Gottingen, but this series is too small to be seriously considered to-day.

III. MATERIAL AND METHOD

The human skeletons of the Hamann Museum collections have been obtained almost entirely from laboratory cadavera. The manner of documentation and preservation, and the demographic characteristics of this skeletal population have been repeatedly described (84, 86). A brief review of these has recently appeared (18). It will suffice at present to cite that for each individual, sex, race, and in most cases, age and birthplace or nationality are known. The whites are chiefly European immigrants or their immediate descendants. The Negroes are mostly industrial migrants from the south. Analysis has shown that this laboratory group presents less evidence of hybridization than the American Negro as a whole (86). The sex, age and racial distribution of the sterna used in this study are furnished in Table IX. The anthropoid sterna were from animals secured in their native habitats.

The roentgenograms and dry bones of Series I were studied first. The dissections were made after the specimens had been roentgenographed. This experience furnished the background necessary for the classification of the specimens in Series II which consisted of bones only. Here often the presence of suprasternal articular facets was accepted as reliable evidence of the existence of separate ossicles which had been lost.

The drawings of Figs. 1 and 4 were traced in outline from the roentgenograms and embellished from observation of the actual specimens. The superior aspects of the upper manubrial margins seen in Fig. 3 are camera lucida drawings of the bones themselves.

IV. RESULTS OF THE STUDY AND DISCUSSION

The results of the study of Series I and II are presented in the figures and tables. The findings from Series III and the dissections are incorporated in the discussion.

Variation

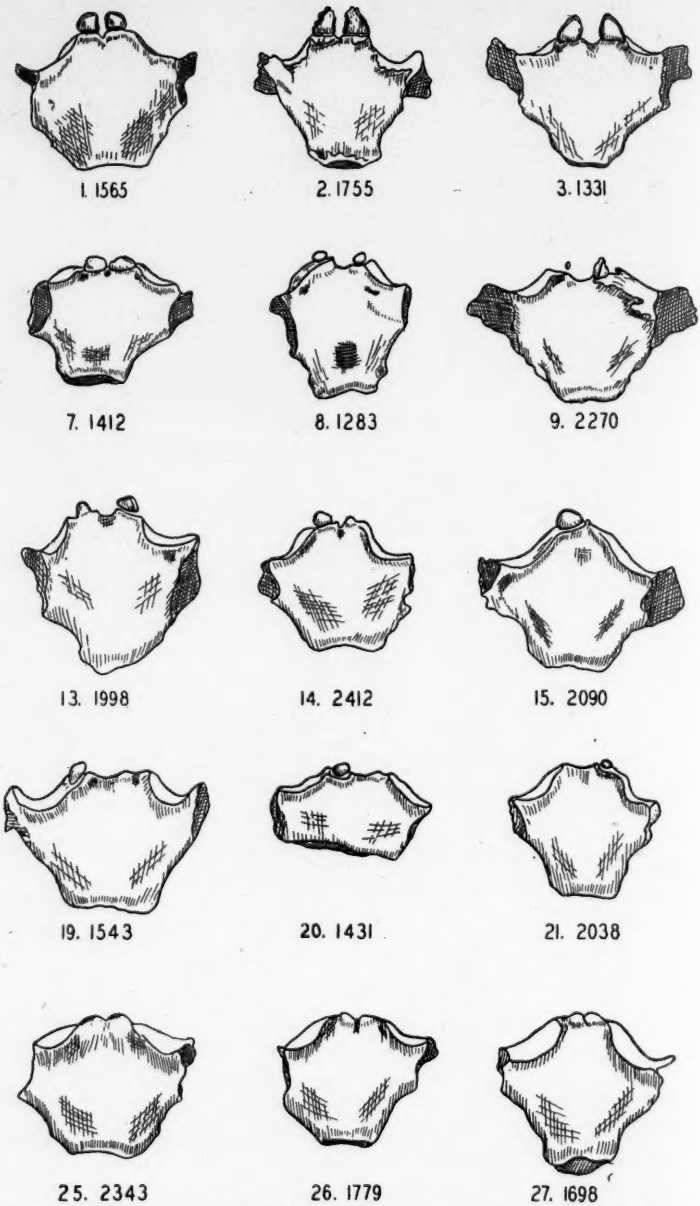
The specimens of Figs. 1 and 2 illustrate the variation of suprasternal bones in size, form, position and integrity. They may occur in pairs or singly. They may be separate, forming a diarthrodial joint with the manubrium complete with articular cartilage and synovial membrane; they may be united to the manubrium by fibrocartilaginous synchondrosis; or as small nodules they may be ensconced within the suprasternal ligaments and have no contact with the manubrium (Fig. 1, no. 9).

The ossicles may exhibit various degrees of fusion with the manubrium and with each other, almost complete incorporation in the former being sometimes detectable (Fig. 1, no. 26). No case was found in which the ossicles had fused with each other but not with the manubrium, although such a case has been reported by Paterson (67).

Paired suprasternal bones may be of subequal size and symmetrical form and position on the manubrium (Fig. 1, nos. 1–4), or of unequal size and asymmetrical arrangement (nos. 9, 10). One element may be separate and the other fused (nos. 11–14).

Size. Suprasternal bones range in size between that of a small shot and an average female lunate bone. The smallest specimen (Fig. 1, no. 9, left) had a height of 4 mm. and a breadth of 2·25 mm. (measured on the roentgenogram). The largest in our series, from cadaver No. 2549, measured 13·5 mm. in length (antero-posterior), 19·5 mm. in breadth, and 10 mm. in height. This slightly exceeds in size the largest specimen previously recorded, which was described by von Eggeling as having a length of 20 mm., a breadth of 11 mm. and a height of 6 mm. Inspection of Fig. 1 readily indicates that a mathematical average size would have little value because of the range and amount of variability, hence the dimensions of 19 separate ossicles of various sizes are presented in Table I.

Form. In the shape of suprasternal bones there is manifest a characteristic basic pattern, a fact which the wide variations in size, symmetry and sharpness of form tend to mask. In its full expression the fundamental pattern is an irregular pyramid with a base, four surfaces and an apex. This is best illustrated in our series by the specimens from No. 1440 (Fig. 2, no. 1). The relationships of the surfaces in the recent state will shortly be described.



N.B. The subscribed number of all figures is the file number of the skeleton in the Hamann Museum.

Fig. 1. Variation of suprasternal bones. Nos. 1–10, paired separate ossicles; nos. 11–14, paired ossicles, one separate, one fused; nos. 15–23, single separate ossicles; nos. 24–27, 30, paired fused ossicles; nos. 28, 29, single fused ossicles.

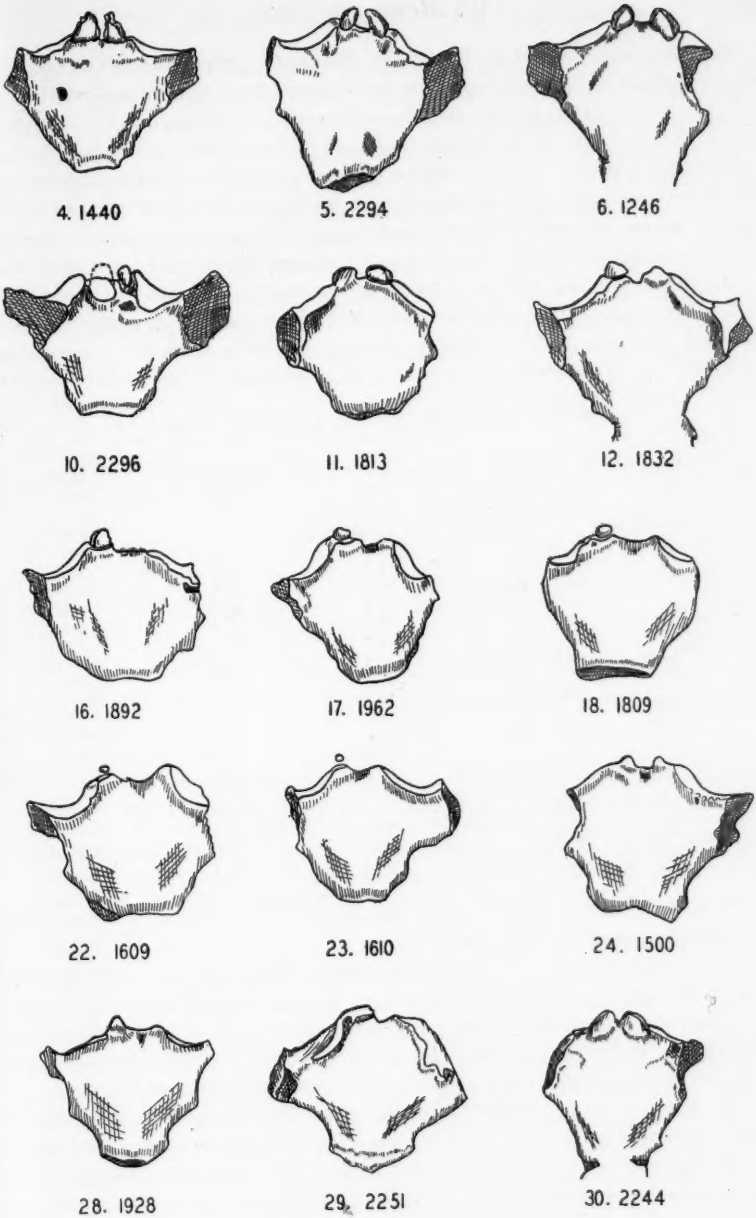


Fig. 1 (*continued*).

The base may be flat with a slight downward projection at the postero-lateral angle, but is usually more or less concave from before backward so as to grip the manubrial surface and in diarthrodial joints permit slight gliding motion. The borders of the base correspond to the surfaces of the ossicle.

The four typical surfaces, anterior (ventral), posterior (dorsal), medial and lateral are represented diagrammatically in Fig. 2. They are separated by borders which converge from the base upon the apex and may be termed respectively antero-medial, antero-lateral, postero-lateral and postero-medial.

Of the surfaces the lateral is the most constant, often the largest, and the medial the smallest and most variable. The more vertical anterior surface is regularly narrower than the sloping convex posterior surface. A similar relationship exists between the narrower more vertical medial surface and the

Table I. *Dimensions of suprasternal bones*

No.	Cadaver No.		Length (antero-posterior) mm.	Breadth mm.	Height mm.
1	2549	R.	13·5	19·5	10·0
		L.	16·25	11·0	10·0
2	2710	R.	9·75	13·0	8·0
		L.	11·5	13·0	7·5
3	1331	R.	10·5	11·5	9·0
		L.	11·0	11·25	9·5
4	2705	R.	9·75	13·0	8·0
		L.	11·5	13·0	7·5
5	1440	R.	11·0	10·0	8·75
		L.	11·0	11·75	8·0
6	1246	R.	12·0	12·5	7·0
		L.	11·0	10·0	7·0
7	2270	R.	12·0	7·0	7·0
		L.	(X-ray)	2·25	4·0
8	1283	R.	6·0	6·5	3·75
		L.	5·25	6·0	3·5
9	1813	R.	11·0	13·0	8·0
		L. (f.)	10·5	9·75	8·0
10	1610	I.	3·5	4·0	3·0
		Average	10·4	10·4	7·3

broad lateral surface which has topographical continuity with the adjacent clavicular notch of the sternum.

The apex is usually definitely recognizable. It is most frequently rounded (Fig. 2, no. 2), but may be flattened (no. 5), or compressed into a sharp or rounded ridge extending either medio-laterally (no. 3), or antero-posteriorly (no. 6). The differences in the relations and sizes of the surfaces associated with the several types of apices are shown by the diagrams of Fig. 2.

The most common variant from the pyramid with quadrangular base is represented by Fig. 2, no. 2, in which the base is triangular, the medial surface here being reduced to a broad rounded border. This may be termed the medial border and be considered to represent the confluence of antero-medial and postero-medial borders.

In even the small decidedly rounded ossicles (Fig. 1, nos. 8, 23) this triangular pyramidal form may be clearly distinguished, especially with

the aid of a hand lens. The antero-posterior elongation seen in the specimen from No. 2270 (Fig. 2, no. 6, right), resulting in elimination of anterior and posterior surfaces is rare. In the specimens of Fig. 2, no. 4, each anterior and the left lateral surfaces have become subdivided by secondary ridges.

In ossicles fused or partially incorporated with the manubrium both four (No. 825) and three (No. 1507) sided pyramidal shapes may be distinctly recognized, the latter being the more common. The quadrilateral form is usual in paired ossicles which are fused to each other as well as the manubrium.

Viewed *in situ* from behind, the silhouettes of well-developed suprasternal bones resemble right triangles of which the bases rest upon the manubrium and the hypotenuses form the lateral borders, the medial borders approaching

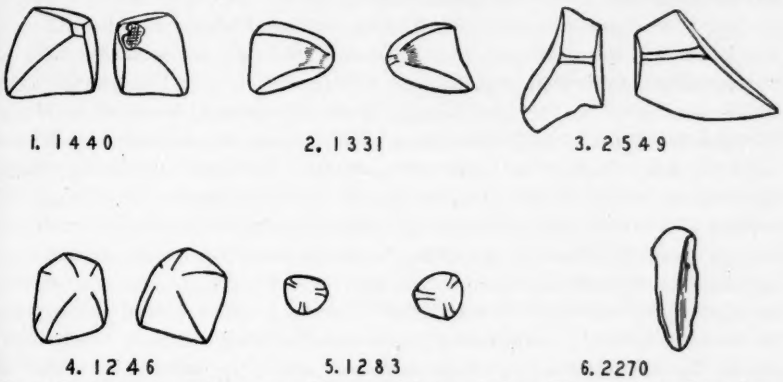


Fig. 2. Typical surfaces of suprasternal bones and their variations. Diagrams drawn from above, ossicles *in situ*, 1½ times natural size. Nos. 1, 3, 4, quadrilateral pyramids. In no. 4, both anterior and the left lateral surfaces are subdivided by secondary ridges. Nos. 2, 5, triangular pyramids. The medial surface is here compressed into a medial border. No. 6, irregular form exhibiting only medial and lateral surfaces. No. 1, blunt apices; nos. 2, 4, 5, rounded apices; no. 3, apex a transverse ridge; no. 6, apex an antero-posterior ridge.

the perpendicular (Fig. 1, nos. 1, 2, 3, 4, 5, 16). The less frequent flattened pyramids may bear resemblance to a biscuit (Fig. 1, nos. 7, 11, 15).

Position and relations. Ossicles of all types occupy a constant position on the posterior portion of the superior manubrial border. The manubrial facets adjoin and many times are confluent with the clavicular articular surfaces (Fig. 3). In other cases the suprasternal facet on the manubrium may be separated from the clavicular articular surface by a distinct groove (Fig. 3, no. 3). The surface of these facets on the manubrium, which are invariably present with well-developed ossicles, does not conform closely to that of the base of the suprasternal bones. The circumference of the facets is of more rounded outline than the base of the suprasternalia. The facets are with few exceptions broader in the lateral than in the antero-posterior direction. The larger facets generally present a central depression, the circumference appearing

as a rim (Fig. 3, no. 1). The surface of these facets is very variable, however. Sometimes it is concave only from side to side. In other instances it is convex antero-posteriorly. The facets are usually supported upon bony shoulders or pedestals which extend medially from the clavicular notches and to varying degree fill the jugular notch. In Fig. 1, no. 15, is seen a well-developed suprasternal bone upon a manubrium with markedly convex upper border. Here the manubrial facet is convex in all directions.

To clarify the further relationships of the ossicles dissections of two roentgenographed sterna bearing paired suprasternal bones were made (Nos. 2710 and 2705). These dissections revealed quite definitely the adaptation of the external form of the suprasternalia to their ligamentous investment and the interarticular disc of the sternoclavicular joint.

No. 2710 presented well-developed suprasternal bones of subequal size. The left ossicle was separate. It was joined to the sternum by a thin layer of fibrocartilage, the union thus being a synchondrosis. The base of the right ossicle was fused to the manubrium. Both suprasternal bones were of the triangular pyramidal type illustrated by Fig. 2, no. 2, and had well-defined surfaces. The anterior and posterior surfaces were each covered by strong ligamentous bands which joined the ossicles to the sternum. These might be termed the anterior and posterior suprasternal ligaments. They conform to the arrangement described by Luschka. Medially these ligaments became thin and blended with weaker ligamentous fibres which linked the rounded medial borders of the two bones to each other. Laterally the suprasternal ligaments blended respectively with the anterior and posterior sternoclavicular ligaments. To the entire lateral surface of each ossicle the interarticular disc of the sternoclavicular joint was firmly attached. The interclavicular ligament proper was easily separated from the fibres joining the bones to each other and passed above the suprasternal ossicles on each side, appearing to have no functional relation to them.

In the other specimen dissected (No. 2705) both ossicles had the triangular pyramidal form and were of subequal size. The disposition of the ligaments was the same as in the previous specimen. Anterior and posterior surfaces were covered by the corresponding suprasternal ligaments, and the broad medial borders were joined by thin strands of weaker fibres. Again the whole lateral surface of each ossicle constituted an impression for the attachment of the fibrocartilaginous meniscus.

The base of the left suprasternal bone articulated with the manubrium by a diarthrodial joint. The articular cartilage was limited to the contiguous surfaces and the synovial cavity had no communications. The right osselet though similar in size and shape to its mate was incompletely ossified. The roentgenogram revealed a pyramidal centre of ossification somewhat greater than half the size of the enclosing cartilage. As the individual in this case was 45 years of age it is believed that we are dealing with mature rather than incomplete development. The hyaline cartilage was united to the manubrium by synchondrosis.

Our findings on the relations of the suprasternal bones generally confirm and in some aspects expand and clarify the descriptions of earlier writers. The existence of definite surfaces and form previously mentioned and detailed by Luschka, in the accurate description of Anthony, but quite obvious in the plates of Breschet, von Eggeling and Dixon, has been shown to be constant, and recognizable even in the smaller and more rounded ossicles. Our dissections have shown that the anterior surface is regularly in relation with the thickening of the investing capsule called the anterior suprasternal ligament; that the posterior surface is constantly covered with a similar capsular specialization, the posterior suprasternal ligament; that the medial surface, whether it be broad and extensive or reduced to a mere rounded border, is joined to the corresponding surface of the companion ossicle by weaker ligamentous fibres which may be called the inter-suprasternal ligament; and that the lateral surface of the ossicles is contiguous with the interarticular disc of the sterno-clavicular joint. The interclavicular ligament was distinct as such and not a proper ligament of the suprasternalia.

These ligamentous arrangements agree essentially with those described with various emphasis by Luschka, Carwardine, Anthony, Lickley, Stein and Dixon. Of earlier writers Dixon alone specifically stated that the suprasternal bones were not connected with the fibrocartilaginous disc. Other authors reported definite connexion between the meniscus and the osselets. On each of the four sides in our two cases the disc was fused with the entire lateral surface of the suprasternal bones. When the disc was torn away, fragments of fibrocartilage remained adherent to the ossicles. In Anthony's case the menisci articulated with the suprasternal ossicles by diarthrodial joints. Dixon's finding is inexplicable in the light of other evidence, and it is accepted here that the meniscus articulates with the lateral surface of the ossicles by diarthrosis or synchondrosis and that the attachment is co-extensive with the lateral surface.

Integrity. Suprasternal bones which are incorporated in the manubrium exhibit a range of variation similar to that of the separate ossicles. Often recognition is difficult. Identification is most unmistakable when the fused ossicle is the subequal mate of a separate one (Fig. 1, no. 11). Fused ossicles of large size paired or single are readily perceived (Fig. 1, nos. 28, 30). In the more complete degrees of incorporation indentification of the suprasternal elements is difficult (Fig. 1, nos. 25–27). The line of junction between suprasternal bone and manubrium may be distinct (No. 825) or indistinguishable (No. 1032). Rounded episternal tubercles or ridges are likely to be confused with the shoulders adjacent to the clavicular facets discussed below. Occasionally extension of clavicular surface with arthritic deposit may mask a fused episternal element (Fig. 1, no. 29).

Examination of the superior manubrial aspects of mooted specimens will disclose true suprasternal tubercles in the characteristic position of the separate ossicles on the posterior portion of the margin (Fig. 3, nos. 25–30). It is freely admitted that in a few cases positive determination cannot be made.

It might be suggested that the minute ossicles of Fig. 3, nos. 9 and 23 are not mature suprasternal bones but centres of ossification of suprasternal cartilages not detectable on the roentgenogram. The specimens of no. 9 are from an individual of 55 years, that of no. 23 from one of 65 years. Despite an incomplete knowledge of the time schedule of development of the suprasternalia, there is no evidence that ossification of even these rudimentary structures would not be completed before the age of 30, for before this age all

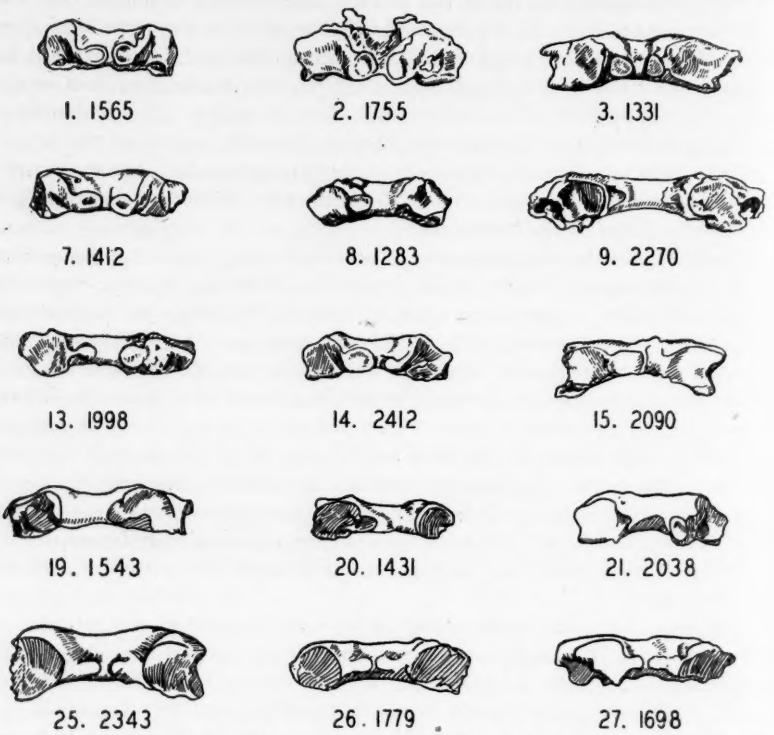


Fig. 3. Vertical aspects of superior manubrial borders. Showing facets for separate ossicles and fused ossicles themselves in constant position on posterior portion of superior border adjoining or adjacent to clavicular facets. Specimens same as in fig. 1.

manifestations of the phenomenon of growth of which the appearance of centres of ossification is but one have disappeared. Moreover, if the nodules were ossific centres in larger cartilages articulating with the manubrium, articular facets would be expected to be present on the manubrium. Examination of the upper manubrial borders of the specimens (Fig. 2 and Fig. 3, nos. 9 and 23), shows that such facets are absent. In addition, the great difference in size of the two ossicles of no. 9 would demand a special explanation if interpreted

as merely differential in developmental progress on the two sides, whereas unequal development of paired rudimentary structures is to be expected. The small ossicles cited, therefore, are regarded as having completed development in their respective individuals.

Structure. The roentgenograms show suprasternal bones to be of the same structure as the sternum. They consist of cancellous tissue invested with a very thin layer of compacta.

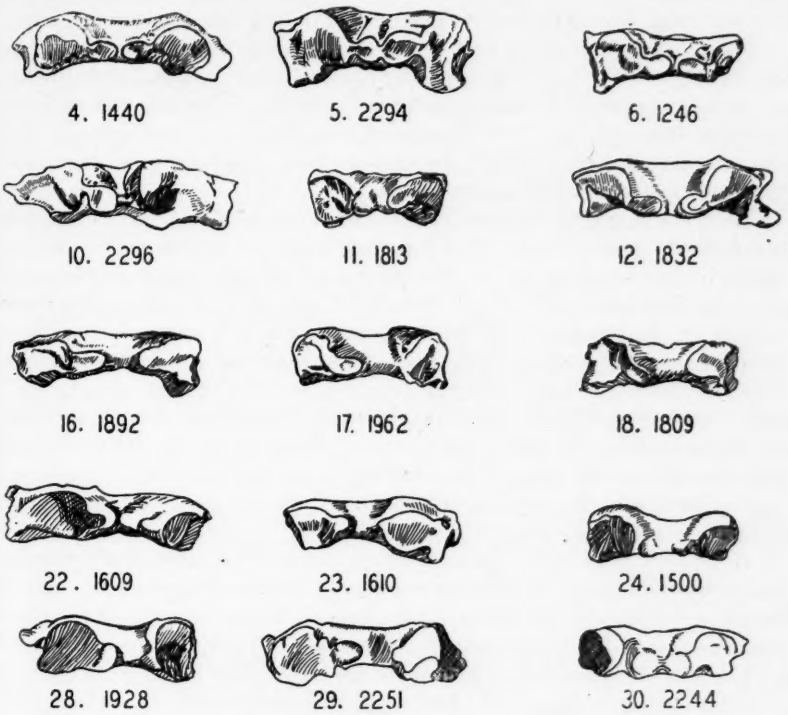


Fig. 3 (*continued*).

Value of the roentgenogram. In consideration of the incidence of the suprasternalia as shown in the tables, only the results of Series I, which consisted of sterna with roentgenograms taken before maceration will be used as final figures. The absence of suprasternal articular facets in the presence of definite suprasternal bones on the manubria of Fig. 3, nos. 9 and 23, as already mentioned, indicates that the dry sterna may yield no evidence of suprasternal bones which existed. It will be noticed also that both the absolute and relative incidence of separate suprasternal bones is greater in the first roentgenographed series than in the larger second series. Accordingly estimates of occurrence

must be based on sterna roentgenographed prior to maceration and not on dry manubria alone, as minute ossicles may be lost from the latter without trace.

Incidence

Classification. The morphological region involved in this study is the superior border of the manubrium between the clavicular notches. This border may be convex, straight, or concave in contour, or it may bear suprasternal ossicles or tubercles. The concave margin is of most frequent occurrence. It is the *incisura jugularis* of the text-books. In a large group of sterna, however, the jugular notch is encroached upon by rounded bony shoulders or buttresses of varying size which extend medially from the clavicular articular surfaces. Sterna of this type have been considered a modification of the concave type and designated "intermediate". In addition there are a few sterna of irregular type such as those of Fig. 4, nos. 16–18.

Both Von Eggeling and Paterson cited the anatomical features of the sterna here termed "intermediate". Von Eggeling called the bony shoulders "tubera jugulare" and found, as we did, that they might be either confluent with the clavicular notch or separated from the latter by a furrow. Von Eggeling was not definite on the significance of these tubera, but he did not appear to think them rudimentary suprasternal bones. Paterson mentioned "projections which formed ridges separated by grooves or facets from the clavicular facets", and, "distinct articular projections separate from or continuous with the clavicular articular surfaces". The articular projections he thought were sometimes for ossicles lost in maceration, but others and the ridges were "associated with variations in the mode of articulation of the clavicle or attachments of the sternoclavicular ligaments".

In dissections of the ligaments of five sterna of the intermediate type as shown by the roentgenograms we found that the bony shoulder or ridge in each case was the site of fixation of the meniscus. The attachment of the interarticular disc to the lateral side of suprasternal tubercles has been described by Luschka and Pansch and figured by the latter. This has also been shown in our dissection of No. 2710. Accordingly, a suprasternal ossicle, using the bony shoulder as a pedestal, merely separates the meniscus from a site of normal attachment.

Fig. 4. A. Variations of manubrial contour and width between clavicular facets. Left column, wide; middle column, medium; right column, narrow. Upper row, markedly convex; second row, slightly convex; middle row, straight; fourth row, slightly concave; bottom row, markedly concave. B. Irregular and intermediate upper manubrial contours. Nos. 16–18, oblique margins; nos. 19–23, encroachment of bony shoulders greater than one-half potential span of jugular notch; nos. 25–28, encroachment less than half span of jugular notch. Nos. 23, 26, 28, 29, line of attachment of clavicular ligaments visible outside of facet and running to medial limit of bony shoulder; nos. 19, 20, 22, 25, 30, bony shoulder and clavicular facet covered by single smooth continuous surface indicating extension of clavicular articular surface. No. 24, unilateral development of bony shoulder.

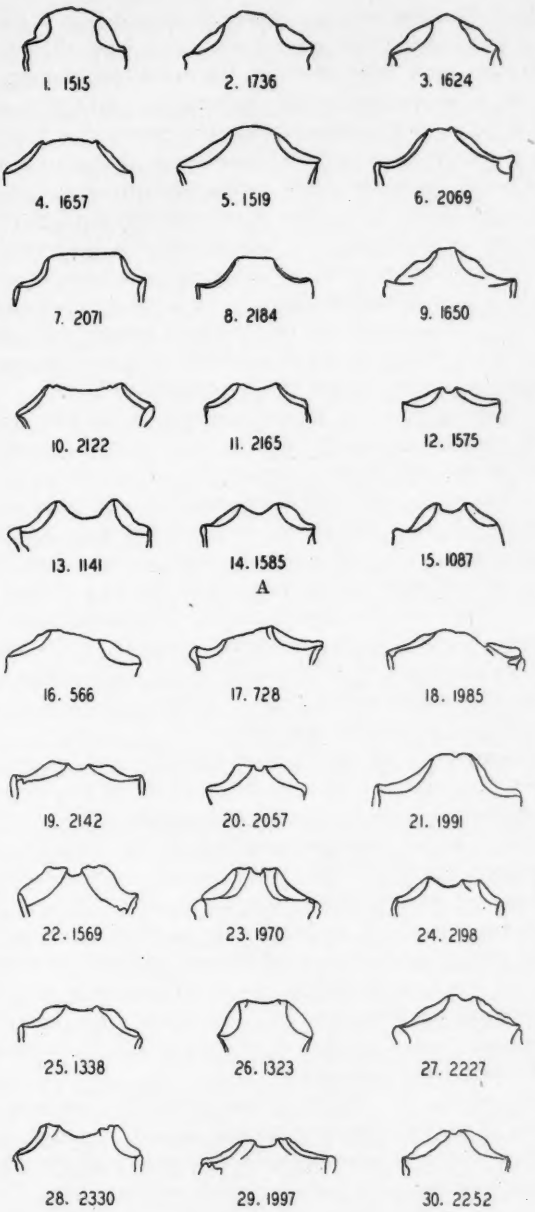

1. 1515
2. 1736
3. 1624
4. 1657
5. 1519
6. 2069
7. 2071
8. 2184
9. 1650
10. 2122
11. 2165
12. 1575
13. 1141
14. 1585
A
15. 1087
16. 566
17. 728
18. 1985
19. 2142
20. 2057
21. 1991
22. 1569
23. 1970
24. 2198
25. 1338
26. 1323
27. 2227
28. 2330
29. 1997
B
30. 2252

Fig. 4.

At this time No. 1283 becomes of special interest (Fig. 1, no. 8). It shows on the left a suprasternal ossicle supported on a bony shoulder or pedestal while on the right there is no shoulder, the ossicle articulating directly with the lateral side of the jugular notch. Nevertheless, on both sides the line of attachment of the sternoclavicular ligaments is perceptible. On the right this line runs up to a faint ridge on the jugular notch, at about the same distance from the adjacent clavicular notch as the breadth of the shoulder on the opposite side. On the left the line blends with the articular edge of the shoulder. Now had the ossicles in this case not been detected by the roentgenogram and preserved, the manubrium itself would have been classed as a unilateral "intermediate", for although the presence of a suprasternal bone on the left side would have been suspected, the articular surface was not sufficiently differentiated to warrant definite assumption, while on the right there was even less suggestion of the presence of a suprasternal bone.

It would thus appear that the attachment of the interarticular disc is marked in the jugular incisura by an impression elevated medially into a faint ridge (Fig. 3, no. 10), and that frequently this area may become raised into a shoulder or transverse ridge which also serves for attachment of the interarticular disc. When the suprasternal bones occur, they intervene between the attachment of the disc and the impressions or shoulders. The shoulders then serve as pedestals for the articulation of the suprasternal ossicles, the discs becoming attached to the lateral surfaces of the latter as has been shown. This relationship might permit suprasternal bones to be described as medial inclusions in the ligaments of the sternoclavicular joint, a slight modification of the concept of Carwardine, who regarded them as occasional inclusions in regularly occurring suprasternal ligaments.

In the present study all suprasternal elements not separate ossicles are included under the designation of suprasternal tubercle. The range of variation of the suprasternal tubercles has already been discussed.

In addition to being classified according to the character of the upper manubrial contour, the sterna were also arranged according to the degree to which the character was expressed. For all types the distance between the clavicular notches was noted as being wide, medium or narrow; convexity and concavity was either slight or marked; and in the intermediate group the encroachment of the bony shoulders was either greater or less than half the span of the potential jugular notch. This classification was purely an arbitrary one, made because it was natural and convenient and might hold the clue to some facts of functional significance.

The tables present the numerical and percentile incidence of the five principal types of upper manubrial border, namely, concave, intermediate, straight, convex, and with suprasternalia. The proper interpretation of these involves certain morphological and functional considerations.

Significance of manubrial types. The fact that evolutionary trend has been toward reduction of the number and size of shoulder-girdle elements at the

cranial end of the presternum would indicate that of the two extreme types of manubrial contour, the markedly convex or the deeply concave, the former is the more primitive.

It is obvious that the convex manubrium has a larger precostal portion than the concave. The significance of this precostal projection is difficult to determine. It may be produced by the incorporation in the manubrium of well-developed suprasternal bones which had fused to each other. Of this the manubria from cadavera Nos. 1440, 825 and 1032 are clear evidence.

No. 1440 (Fig. 1, no. 4) shows separate ossicles in close apposition which, fused to manubrium and each other, would produce a convex border. In No. 825 this fusion has actually occurred, but the lines of demarcation are still discernible. This manubrium is itself small but has relatively large suprasternal bones, and, were fusion complete to the degree manifest in No. 1032, it would be described simply as "convex". In No. 1032, however, small ossicles are incorporated in a large manubrium, and though no lines of fusion are apparent the ossicles are recognizable as a rounded transversely elongated eminence in their characteristic position on the posterior portion of the superior border. Viewed from behind, this manubrium presents a convex outline.

From No. 2090 (Fig. 1, no. 15), on the other hand, we learn that separate suprasternal bones may themselves be supported on a markedly convex manubrial border, and in such instances can have no part in the formation of the latter.

Markowski's figures of foetal and young specimens and the roentgenograms of the young individuals in our own Series III show convex, straight, and concave manubrial margins preformed in cartilage with ossification proceeding from a single manubrial centre. Thus, though a convex manubrial border may be formed by fusion of suprasternal bones, it may also arise independently of the ossicles.

From the comparative standpoint Paterson states that among the Mammals it is much commoner for the presternum to project in front of the first rib than not. He adds that the process may be median or bilobed; that when present it gives attachment to the clavicles; and that it is not characteristically present in sterna of the human type. The convex border may therefore be considered a manifestation of a frequent mammalian tendency for the presternum to project forward. It is conceivable that the human convex manubrium or the projecting manubrium of other Mammals may represent incorporated vestige of the omosternum of the Amphibia, though there is no evidence beyond topographical analogy to support this view.

Functionally the convex or projecting border would appear to provide a stronger basis for the sternoclavicular joint than the concave, with greater protection particularly against inward thrusts. There is slight indirect evidence for this in the higher percentages of intermediate sterna in the Negro as compared with the white. If it be true that the Negro has progressed further in the direction of simplifying structure at the upper end of the sternum, the

shoulders of the intermediate type can be interpreted as a secondary functional reaction to strengthen a weakened joint.

Dissections and statistics on the form of the manubrium from larger series of anthropoids than are at present available should throw more light on the amount of functional adaptation existing in the sternoclavicular joint, inasmuch as the amount of brachiation engaged in by each of these animals is adequately known.

The hypothesis which will be used as a basis for the analysis of our tables of incidence will be, therefore, that the human sternum with convex manubrial border and well-developed separate ossicles retains the greatest number and quantity of primitive shoulder-girdle elements and must be considered the primitive type, while the markedly concave sternum represents the greatest reduction in primitive elements and is hence the most advanced. Interpolated in descending order from advanced to primitive are intermediate, straight, and convex types, it being recognized that the intermediate may be interpreted both as a regressive straight type or a concave in which the notch is secondarily rebuilt.

The dangers of such an hypothesis in the absence of direct evidence is fully realized. Yet our indirect evidence is in a positive direction, and the opposite assumption, that the concave is primitive and the convex advanced, fails of support unless one assume the clavicles to be sliding down the sternum. To dismiss the whole matter as mere normal variation would seem to be dodging the problem.

Frequency of types of ossicles. The individuals from whom the suprasternal bones of this study were obtained are identified in Table II. It is immediately apparent that neither age, origin, cause of death nor associated sternal anomaly suggest that suprasternal bones have any particular constitutional affinity.

The frequency of paired, single, separate and fused ossicles appears in Table III. It will be noticed that the number and percentage of separate ossicles is much greater in Series I than Series II, while the reverse is true of tubercles. The apparent discrepancy is readily explained, first, by the fact already mentioned that the roentgenograms of Series I made possible detection of more ossicles than could be done in Series II, and second, because Series II contained a much larger proportion of male whites. The frequency in whites is greater than in Negroes, as will shortly be seen.

The disparity disappears when the percentages of suprasternal tubercles in the two groups are compared (Table III). These show no significant differences between the two series except in the female whites, who present a higher percentage of tubercles in Series I. The fact that these female white series were very small, numbering 79 and 83 individuals respectively, is to be taken into account.

As in the other tables of the incidence of suprasternal elements, the frequency shown by Series I is held most reliable. Because of the small numbers involved only the male series will be used. In the male whites of Series I,

Table II. *Cadavera with suprasternal ossicles or tubercles*

Sex and race	Cadaver No.	Age	Birthplace	Cause of death	Supra-sternalia*	Additional sternal anomaly or defect
				Series I. With X-rays		
Male, white	1246	39	United States	Cerebral haemorrhage	2 S	Manub., body united
	1331	27	United States	Carbon monoxide poisoning	2 S	None
	1412	31	Unknown	Carcinoma of stomach	2 S	None
	1431	42	United States	Alcoholic poisoning	2 S	None
	1440	79	Germany	Cerebral haemorrhage	2 S	None
	1543	75	United States	Cerebral haemorrhage	2 S	None
	1565	35	Unknown	Acute alcoholism	2 S	Large perforation
	1597	57	Unknown	Empyema of gall bladder	2 S	Sep. sternebra; perforat.
	2270	55	Unknown	Carcinoma of oesophagus	2 S	None
	2294	64	Unknown	Cerebral haemorrhage	2 S	Oblique man.-st. joint
	2299	50	Unknown	Myocarditis	2 S	None
	2457	44	Unknown	Pulmonary carcinoma	2 S	None
	2549	60	Pennsylvania	Arteriosclerosis	2 S	None
	2705	45	Unknown	Lobar pneumonia	2 S	None
	1609	61	Ireland	Auto accident	1 S	None
	1610	65	Italy	Auto accident	1 S	None
	1809	45	Unknown	Suicide	1 S	None
	1892	46	Greece	Pulmonary tuberculosis	1 S	Perforation
	2164	54	Unknown	Auto accident	1 S	None
	2187	37	Russia	Pulmonary tuberculosis	1 S	None
	2296	50	Unknown	Alcoholism	1 S	None
	2710	56	Unknown	Cardiac failure	1 S	None
	1813	66	Unknown	Gastritis	1 S, 1 T	None
	1998	64	Germany	Chronic nephritis	1 S, 1 T	Oblique man.-st. joint
	1500	70	United States	Chronic myocarditis	2 T	None
	1698	76	New York	Pyelocystitis	2 T	None
	2272	48	Unknown	Peritonitis	2 T	None
	2343	50	Unknown	Tuberculous meningitis	2 T	Half perfor. fr. ven. side
	1928	68	Serbia	Chronic myocarditis	1 T	None
	2251	61	Pennsylvania	Bronchial pneumonia	1 T	None
	2345	35	Unknown	Diabetes	1 T	Man. united to body
Female, white	1755	73	Austria	Cerebral haemorrhage	2 S	None
	1369	25	Ohio	Pulmonary tuberculosis	1 S	None
	1779	78	Germany	Bronchial pneumonia	2 T	None
	1938	79	Germany	Diabetes	2 T	Perf.; artic. 6 ribs
	2244	44	Hungary	Paresis	2 T	None (healed frac. corp.)
	1762	61	United States	Manic depressive psychosis	1 T	Very narrow, art. 6 ribs
Male, Negro	1283	23	Georgia	Pulmonary tuberculosis	2 S	None
	1962	42	Unknown	Dropsy	1 S	None
	2038	30	Unknown	Alcoholic poisoning	1 S	None
	2090	42	Alabama	Pulmonary tuberculosis	1 S	Sep. sternebra
	1832	37	Alabama	Coronary thrombosis	1 S, 1 T	Man. united to body
	2412	36	Ohio	Lobar pneumonia	1 S, 1 T	None
	1394	54	Tennessee	Rupture aortic aneurysm	1 T	None
	1507	22	Unknown	Pulmonary tuberculosis	1 T	None
	2005	53	Unknown	Skull fracture	1 T	None
	2016	45	Canada	Cardiac failure	1 T	None
Female, Negro	None					
				Series II. No X-rays		
Male, white	205	37	Austria	Myocarditis	2 S	None
	338	40	Unknown	Cerebral tumour	2 S	Body in two pieces
	395	55	Unknown	Lobar pneumonia	2 S	Perforation
	688	24	Unknown	Tuberculosis	2 S	None

* S, separate ossicle; T, tubercle.

Table II (*continued*)

Sex and race	Cadaver No.	Age	Birthplace	Cause of death	Supra-sternalia*	Additional sternal anomaly or defect
Male, white	255	48	Germany	Gangrene of foot	1 S	None
	746	45	Unknown	Skull fracture	1 S	None
	65	30	Unknown	Gunshot wound	1 S, 1 T	None
	424	26	Russia	Sarcoma	1 S, 1 T	Sl. obliquity man.-corp. joint
	429	40	Germany	Peritonitis	1 S, 1 T	None
	94	28	Unknown	Skull fracture	2 T	None
	104	35	Unknown	Lobar pneumonia	2 T	None
	115	88	Unknown	Nephritis	2 T	Manub. united to body
	383	35	Unknown	Suicide	2 T	None
	689	45	Unknown	Influenza	2 T	Manub. united to body
	701	41	Austria	Tuberculosis	2 T	None
	1032	73	Austria	Paresis	2 T	None
	1038	48	Bohemia	Pulmonary tuberculosis	2 T	None
	1045	49	Bohemia	Lues	2 T	None
	1164	49	Unknown	Pulmonary tuberculosis	2 T	Manub. united to body
	1230	58	Unknown	Skull fracture	2 T	None
	371	35	Unknown	Suicide	1 T	None
	489	64	Unknown	Tuberculosis	1 T	None
	550	39	Unknown	Tuberculosis	1 T	None
	997	40	Russia	Chronic myocarditis	1 T	None
	1013	60	New York	Mitral and aortic stenos.	1 T	None
	1179	35	United States	Alcoholic poisoning	1 T	None
Female, white	774	38	Unknown	Influenza	2 S	None
	631	31	Unknown	Myocarditis	2 T	None
	1066	51	Unknown	Arteriosclerosis	2 T	None
Male, Negro	25	35	Unknown	Unknown	1 S	None
	736	40	Unknown	Myocarditis	2 T	None
	825	21	Virginia	Pneumonia	2 T	None
	1201	29	Alabama	Pulmonary tuberculosis	2 T	Perforation
	778	28	Ohio	Tuberculosis	1 T	None
Female, Negro	868	60	Unknown	Myocarditis	2 S	None

* S, separate ossicle; T, tubercle.

46·5 per cent. of 31 cases of ossicles were paired and separate; 25·8 per cent. single and separate; 6·4 per cent. had one member of a pair separate and the other fused; 12·9 per cent. paired and fused; and 9·7 per cent. single and fused. The ossicles in the 10 male Negroes were paired and separate in 10 per cent.; single and separate in 30 per cent.; 1 separate, 1 fused in 20 per cent.; no fused pairs; but single fused ossicles in 40 per cent. Combining the stocks, out of 47 cases of ossicles in 1010 individuals, 34 per cent. were paired, separate; 25·5 per cent. single, separate; 8·5 per cent. paired, 1 separate, 1 fused; 14·8 per cent. paired, fused; 17 per cent. single, fused.

It may, therefore, be said that about two-thirds of the suprasternal ossicles which occur are separate and one-third fused; that paired ossicles are slightly more frequent than single in the separate group, but are of approximately equal frequency among fused ossicles. Although we are dealing with three times as many cases of ossicles in whites as in Negroes, the greater percentage of paired separate and single separate ossicles in the white indicates that this rudimentary structure attains fuller expression in the latter group, though

Table III. *Incidence of ossa and tubercula suprasternalia*

Type of ossicle	White						Negro						Total					
	Male		Female		Total		Male		Female		Total		Male		Female		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Paired separate:																		
I	14	46·5	1	16·7	15	40·5	1	10·0	—	—	1	10·0	15	36·6	1	16·7	16	34·0
II	4	15·4	1	33·3	5	17·2	—	—	1	100·0	1	16·7	4	12·9	2	50·0	6	17·1
I and II	18	31·6	2	22·2	20	30·3	1	6·7	1	100·0	2	12·5	19	26·8	3	30·0	22	26·8
Single separate:																		
I	8	25·8	1	16·7	9	24·3	3	30·0	—	—	3	30·0	11	26·8	1	16·6	12	25·5
II	2	7·7	—	—	2	6·9	1	20·0	—	—	1	16·6	3	9·6	—	—	3	8·5
I and II	10	17·5	1	11·1	11	16·7	4	26·7	—	—	4	25·0	14	19·4	1	10·0	15	18·3
Separate and fused:																		
I	2	6·4	—	—	2	5·4	2	20·0	—	—	2	20·0	4	9·7	—	—	4	8·5
II	3	11·5	—	—	3	10·3	—	—	—	—	—	—	3	9·6	—	—	3	8·5
I and II	5	8·8	—	—	5	7·6	2	13·3	—	—	2	12·5	7	9·7	—	—	7	8·5
Paired fused:																		
I	4	12·9	3	50·0	7	18·9	—	—	—	—	—	—	4	9·7	3	50·0	7	14·8
II	11	42·3	2	66·7	13	44·8	3	60·0	—	—	3	50·0	14	45·1	2	50·0	16	45·7
I and II	15	26·3	5	55·5	20	30·3	3	20·0	—	—	3	18·7	18	25·0	5	50·0	23	28·0
Single fused:																		
I	3	9·7	1	16·7	4	10·8	4	40·0	—	—	4	40·0	7	17·1	1	16·7	8	17·0
II	6	23·1	—	—	6	20·7	1	20·0	—	—	1	16·7	7	22·6	—	—	7	20·0
I and II	9	15·8	1	11·1	10	15·1	5	33·3	—	—	5	31·2	14	19·4	1	10·0	15	18·3
Total:																		
I	31	100·0	6	100·0	37	100·0	10	100·0	—	—	10	100·0	41	100·0	6	100·0	47	100·0
II	26	100·0	3	100·0	29	100·0	5	100·0	1	100·0	6	100·0	31	100·0	4	100·0	35	100·0
I and II	57	100·0	9	100·0	66	100·0	15	100·0	1	100·0	16	100·0	72	100·0	10	100·0	82	100·0
Percentages of tubercula suprasternalia (fused, paired and single)																		
I	1·5		5·0		2·0		1·2		0·0		0·9						1·5	
II	2·4		2·4		2·4		1·8		0·0		1·1						2·0	

this, of course, cannot be said of the form and development of individual ossicles.

Frequency of ossicles. The incidence of suprasternal ossicles and tubercles in our several groups is shown in Table IV. These results are compared with those of other investigators in Table V.

Again using the figures of Series I, it is seen that ossa or tubercula suprasternalia occurred in 6·8 per cent. of 544 adult whites and 2·2 per cent. of 466 adult Negroes. Incidence was highest in 79 white females, 7·6 per cent., lowest in 132 Negro females, 0·0 per cent. (Note that one pair of separate ossicles was found in a Negro female in Series II.) For the males the percentages were: 465 whites, 6·7; 334 Negroes, 3·0. Thus the suprasternal elements appear three times more frequently in whites than in Negroes. They are more than twice as frequent in white males than in Negro males, and nearly eight times more frequent in white females than in Negro females.

A difference in racial incidence so marked cannot fail to be striking when the small size of the female samples is considered. It is more arresting in the face of the hybridization which has certainly affected the Negro sample, though, as has been mentioned, hybridization has not been manifest in the physical characters of the laboratory sample to the same extent as in the American Negro as a whole.

Frequency of manubrial types. Returning to our hypothesis that the convex manubrium with well-developed suprasternal bones is the fullest expression in Man of the primitive elements of the shoulder girdle and the markedly concave the most removed from the primitive condition, it is significant to find, in addition to an incidence of suprasternal bones three times greater in whites than in Negroes, that the convex and straight manubrial borders also occur in greater percentage in whites than in Negroes, while the concave form is 7·5 per cent. more frequent in Negroes, both concave and intermediate types being grouped together as concave. For manubrial type both Series I and II were used, a total of 2153 individuals, there being no objection to this procedure here.

The incidence of the suprasternalia and the manubrial types thus seem to indicate a racial difference in development away from the primitive type of presternal end, the Negro having progressed further in this direction than the white.

Anthropoids. Table X shows the incidence of the several manubrial types in the anthropoid series of 61 gorillas, 38 chimpanzees and 8 orangs. No suprasternal ossicles or tubercles were found. The numbers of each animal are, of course, too small to permit any definite suggestion as to their occurrence in these Primates. It is of interest to note that 74 per cent. of the gorillas, 74 per cent. of the chimpanzees, and 62·5 per cent. of the orangs had concave borders, and that no gorillas or chimpanzees and but one orang had convex borders. This would imply further progress than Man has made away from the primitive type of sternum, an entirely plausible possibility, because in

Table IV. *Types of manubria according to sex and race*

	White			Negro			Total		
	♂	♀	Total	♂	♀	Total	♂	♀	Total
Convex	31	6	37	10	0	10	41	6	47
Straight	283	59	342	183	39	222	466	98	564
Concave	151	14	165	141	93	234	292	107	399
Total	465	79	544	334	132	466	799	211	1010

Table IV. *Types of manubria according to sex and race*

	White						Negro						Total					
	Male		Female		Total		Male		Female		Total		Male		Female		Total	
Series	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Concave																		
I	241	51·9	41	51·2	282	51·8	160	47·9	62	47·0	222	47·6	401	50·2	103	48·8	504	49·9
II	401	57·8	41	49·4	442	56·9	151	56·7	44	61·9	195	57·8	552	57·5	85	55·2	637	57·2
I and II	642	55·4	82	50·6	724	54·8	311	51·8	106	52·2	417	51·9	953	54·2	188	51·5	1141	53·7
Intermediate																		
I	109	23·4	15	18·9	124	22·7	120	35·9	49	37·1	169	36·3	229	28·6	64	30·3	293	29·0
II	147	21·2	18	21·7	165	21·3	74	27·8	16	22·5	90	26·7	221	23·0	34	22·1	255	22·9
I and II	256	22·1	33	20·4	289	21·9	194	32·3	65	32·0	259	32·2	450	25·6	98	26·8	548	25·8
Straight																		
I	52	11·2	16	20·2	68	12·5	40	11·9	17	12·9	57	12·2	92	11·6	33	15·6	125	12·4
II	85	12·3	20	24·1	105	13·5	26	9·8	7	9·8	33	9·8	111	11·6	27	17·5	138	12·4
I and II	137	11·8	36	22·2	173	13·1	66	11·0	24	11·8	90	11·2	203	11·6	60	16·4	263	12·4
Convex																		
I	32	6·9	1	1·2	33	6·1	4	1·2	4	3·0	8	1·7	36	4·5	5	2·4	41	4·1
II	34	4·9	1	1·2	35	4·5	10	3·7	3	4·2	13	3·8	44	4·6	4	2·6	48	4·3
I and II	66	5·7	2	1·2	68	5·1	14	2·3	7	3·5	21	2·6	80	4·5	9	2·5	89	4·2
Ossa or tubercula suprasternalia																		
I	31	6·7	6	7·6	37	6·8	10	3·0	0	0·0	10	2·2	41	5·1	6	2·8	47	4·6
II	26	3·7	3	1·8	29	3·7	5	1·9	1	1·4	6	1·8	31	3·2	4	2·6	35	3·1
I and II	57	4·9	9	5·5	66	5·0	15	2·5	1	0·5	16	2·0	62	4·1	10	2·7	82	3·9
Total																		
I	465	85·5	79	14·5	544	53·9	334	71·7	132	28·3	466	46·1	799	79·1	211	20·9	1010	47·6
II	693	89·3	83	10·7	776	69·7	226	78·4	71	21·6	337	30·3	959	86·2	154	13·8	1113	52·4
I and II	1158	87·7	162	12·3	1320	62·2	600	74·7	203	25·3	803	37·8	1758	82·8	365	17·2	2123	100·0

Table V. *Incidence of ossa suprasternalia and manubrial types according to various investigators*

		Spec. with separate ossicles		Spec. with tubercles only		Spec. with ossa or tubercles		Superior manubrial margin					
								Convex		Straight		Concave*	
Series	No. sterna in series	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Strauch	200	5	2·5	6	3·0	11	5·5	—	—	—	—	—	—
Paterson	563	1	0·2	44	7·8	45	7·9	35	6·2	16	2·8	467	82·9
Bogusat	120	3	2·5	—	—	3	2·5	—	—	—	—	—	—
Eggeling	226	9	4·0	21	9·3	30	13·2	53		23·4		144	64·6
Barchielli	162	0	0·0	3	1·8	3	1·8	7	4·3	43	26·5	109	67·2
W.R.U., I	1010	33	3·3	14	1·3	47	4·6	41	4·1	125	12·4	797	78·9
W.R.U., II	1113	12	1·1	23	2·0	35	3·1	48	4·3	138	12·4	892	80·1
W.R.U., I and II	2123	45	2·2	37	1·7	82	3·9	89	4·2	203	12·4	1689	79·5

* Includes both concave and intermediate types.

many characteristics of the higher Primates one or more of the great apes has attained more advanced development than that found in Man.

The higher Primates have relatively short and broad sterna as contrasted with the lower. Schultz(77) has shown that siamang, orang and gorilla have each progressed further in this direction than has Man. Nor is it found that the genus which shows the most advanced development in one of the higher Primate characters exhibits similar advancement in all. Thus although the chimpanzee manifests to a marked degree the tendency to fusion of the segments of the corpus sterni most fully realized in Man, yet in relative breadth the sternum of the chimpanzee is not much greater than that of the lower Primates. Similarly, though the orang has attained a tremendously short and broad sternum, the segments of the corpus tend to remain ununited, except the most caudal.

Our findings in the region of the hafting of the shoulder girdle are thus in full accord with Primate tendencies in development as revealed by evidence now available. There is no reason to expect in other regions or relationships of the human sternum correlated advanced or primitive stages. Unfortunately the literature does not yield at present adequate information on the morphology of the human sternum. Those series which have been studied are on the whole too small to furnish satisfactory knowledge of the variations and their incidence, racial and otherwise, of this highly variable bone.

Width of superior manubrial border. In surveying the aspects of the subject which should be explored in an investigation of suprasternal bones, it appeared that the proximity of the clavicular facets as measured by the distance separating them might have possible functional significance. Accordingly an attempt was made to obtain information on this phase by classifying each upper manubrial border as being relatively wide, medium or narrow. The results appear in Tables VI and VII, illustrated by fig. 4, which show wide, medium and narrow varieties of the several types of manubrial contour. These appraisals of width are empirical commonsense estimates, undoubtedly neither so desirable nor as accurate as indices derived from actual measurement of the superior borders and the manubria themselves, but still possessing a practical value.

Examination of Table VII, which shows the number and percentages of the widths irrespective of the type of contour, reveals no racial and but slight sexual difference. Of the combined series 34·5 per cent. were classed as wide, 52·7 per cent. as medium and 12·7 per cent. as narrow in distance between their clavicular articular surfaces. The males show approximately 12 per cent. more borders with widely spaced articular surfaces than the females, while the white females have 7 per cent. more borders with narrow-spaced surfaces than the white males. The proportion of narrow borders between the sexes is nearly the same in the Negro series.

From this it may be said that the distance along the superior manubrial border between the clavicular articular surfaces has a greater tendency to be

Table VI. *Distribution of sterna according to contour of superior manubrial border, sex and race*

Contour of manubrium		Distance between clavicular articular surfaces: Wide, Slight		Wide, Marked		Medium, Slight		Medium, Marked		Narrow, Slight		Narrow, Marked		Total: Series I	Total: Series II
Convex:															
White:	Male	12	4	6	3	7	12	5	8	2	4	—	3	32	34
	Female	—	—	—	—	1	—	—	1	—	—	—	—	1	1
Negro:	Male	1	2	—	—	1	2	2	4	—	—	—	2	4	10
	Female	2	—	1	—	1	—	—	2	—	1	—	—	4	3
	Total	15	6	7	3	10	14	7	15	2	5	—	5	41	48
Straight:		Series I		Series II		Series I		Series II		Series I		Series II			
White:	Male	25		37		21		42		6		6		52	85
	Female	7		7		8		11		1		2		16	20
Negro:	Male	17		7		17		17		6		2		40	26
	Female	8		—		8		6		1		1		17	7
	Total	57		51		54		76		14		11		125	138
Concave*:		Deep		Shallow		Deep		Shallow		Deep		Shallow			
White:	Male	31	21	75	69	54	118	52	124	9	33	20	36	241	401
	Female	—	1	8	6	11	6	12	15	1	2	9	11	41	41
Negro:	Male	20	8	47	18	33	44	27	50	16	18	17	13	160	151
	Female	1	—	20	1	13	12	16	21	6	3	6	7	62	44
	Total	52	30	150	94	111	180	107	210	32	56	52	67	504	637
Intermediate:		En $<\frac{1}{2}$ N		En $>\frac{1}{2}$ N		En $<\frac{1}{2}$ N		En $>\frac{1}{2}$ N		En $<\frac{1}{2}$ N		En $>\frac{1}{2}$ N			
White:	Male	58	34	23	11	10	65	17	32	—	2	1	3	109	147
	Female	4	2	4	1	4	9	2	4	1	2	—	—	15	18
Negro:	Male	48	17	21	3	21	35	29	17	1	2	—	—	120	74
	Female	10	2	2	—	18	9	16	4	3	—	—	1	49	16
	Total	120	55	50	15	53	118	64	57	5	6	1	4	293	255
													Total	963	1078

* May show small tubercles for ligamentous attachment but no bony shoulders.

† Bony shoulders or tubera encroach upon jugular notch.

N.B. The first column under each heading refers to Series I and the second column to Series II.

wide relative to the breadth of the manubrium in males than in females. The meaning of this tendency is not now obvious. The anthropoid data are too limited to permit of functional comparisons based on the brachiating habits of the apes and the generalized use of the upper limb in Man. The data as further subdivided in Table VI shed no additional light on this phase. Hence, there appears no association between the width of the superior manubrial margin and suprasternal bones such as existed between the latter and the contour of the manubrial margin.

Age incidence of ossa suprasternalia and manubrial types. The 82 suprasternal ossicles and tubercles encountered in this study all occurred in adults. In the roentgenograms of our 65 young subjects no suprasternal elements, ossified or cartilaginous, were found.

Table VII. *Incidence of wide, medium and narrow upper manubrial borders*

	Wide		Medium		Narrow		Total	
Sex and race	No.	%	No.	%	No.	%	No.	%
White: Male	409	37·1	567	51·5	125	11·4	1101	100·0
Female	40	26·1	84	54·9	29	18·9	153	100·0
Negro: Male	209	35·7	299	51·1	77	13·2	585	100·0
Female	47	23·3	126	62·4	29	14·3	202	100·0
Total	705	34·5	1076	52·7	260	12·7	2041	100·0

The age curves of the subjects having suprasternal bones are not dissimilar from those of the series themselves as plotted from the data of Tables VIII and IX respectively, so that the median or percentage age incidence are of no value. The youngest subject in our series having suprasternal bones was No. 825, a male Negro of 21 years. The specimens were fused but well developed as previously described (p. 265). There were in all ten cases of suprasternalis in the third decade.

The developmental history of suprasternal bones in the light of present evidence may be briefly stated as follows. Suprasternal bones originate in two lateral condensations of mesoderm from the median anlage of the sternum, independent of the sternal bands and ribs. The anlagen become cartilaginous. Suprasternal cartilages have been found in foetuses of 6 months (Paterson). These may persist into adult life (Breschet). Ossification has been observed earliest at 12½ years (von Eggeling). It is probable that ossification generally occurs toward the end of the second decade as part of the last wave of maturation processes which includes epiphysial union and the ossification of vertebral, costal and clavicular epiphyses. The ossification of the cartilage is usually complete but may be partial.

The age incidence of the several types of manubrial contour was plotted, but no type exhibited a particular age incidence such as Graves has found for the vertebral border of the scapula.

Associated anomaly. Since Carwardine, Lickley and Leboucq thought suprasternal bones to be associated with or a manifestation of defective osseous

Table VIII. *Age incidence of ossa suprasternalia*

	White									Negro											
	Male			Female			Total			Male			Female			Total			Total		
Age	I	II	I and II	I	II	I and II	I	II	I and II	I	II	I and II	I	II	I and II	I	II	I and II	I	II	I and II
20–29	1	3	4	1	—	1	2	3	5	2	3	5	—	—	—	2	3	5	4	6	10
30–39	5	6	11	—	2	2	5	8	13	3	1	4	—	—	—	3	1	4	8	9	17
40–49	6	11	17	1	—	1	7	11	18	3	1	4	—	—	—	3	1	4	10	12	22
50–59	7	2	9	—	1	1	7	3	10	2	—	2	—	—	—	2	—	2	9	3	12
60–69	8	2	10	1	—	1	9	2	11	—	—	—	—	1	1	—	1	1	9	3	12
70–79	4	1	5	3	—	3	7	1	8	—	—	—	—	—	—	—	—	—	7	1	8
80–89	—	1	1	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	1	1
Total	31	26	57	6	3	9	37	29	66	10	5	15	—	1	1	10	6	16	47	35	82

Table IX. *Distribution of sterna according to age by decade, sex, race, and contour of superior manubrial border*

	White														Negro													
	Male					Female					Total					Male					Female					Total					Total W and N				
Age	Cv.	St.	Cc.	In.	Total	Cv.	St.	Cc.	In.	Total	Cv.	St.	Cc.	In.	Total	Cv.	St.	Cc.	In.	Total	Cv.	St.	Cc.	In.	Total	Cv.	St.	Cc.	In.	Total	Cv.	St.	Cc.	In.	Total
Series I. Sterna with roentgenograms																																		
10–19	—	—	1	—	1	—	—	1	1	2	—	—	2	1	3	—	—	1	—	1	—	—	1	1	2	—	—	2	1	3	—	—	4	2	6
20–29	1	1	6	5	13	—	1	1	—	2	1	2	7	5	15	—	7	25	18	50	2	1	18	16	37	2	8	43	34	87	3	10	50	39	102
30–39	1	7	17	5	30	—	—	5	2	7	1	7	22	7	37	—	4	42	29	75	—	7	18	9	34	—	11	60	38	109	1	18	82	45	146
40–49	9	11	61	25	106	—	4	11	4	19	9	15	72	29	125	2	15	38	28	83	—	3	10	11	24	2	18	48	39	107	11	33	120	68	232
50–59	10	16	71	34	131	—	1	10	2	13	10	17	81	36	144	1	7	28	28	64	2	2	6	5	15	3	9	34	33	79	13	26	115	69	223
60–69	10	14	50	24	98	1	4	5	1	11	11	18	55	25	109	1	4	17	11	33	—	3	5	2	10	1	7	22	13	43	12	25	77	38	152
70–79	1	2	27	12	42	—	3	4	3	10	1	5	31	15	52	1	2	6	3	12	—	2	4	2	8	1	4	10	5	20	2	9	41	20	72
80–89	—	1	8	4	13	—	3	4	1	8	—	4	12	5	21	—	1	2	2	5	—	—	1	1	2	—	1	3	3	7	—	5	15	8	28
90–99	—	—	—	—	—	—	—	—	1	1	—	—	—	1	1	—	—	1	—	1	—	—	—	—	—	—	—	1	—	1	—	—	1	1	2
Total	32	52	241	109	434	1	16	41	15	73	33	68	282	124	507	5	40	160	119	324	4	18	63	47	132	9	58	223	166	456	42	126	505	290	963
Series II. Bones only																																		
10–19	—	—	3	—	3	—	—	2	—	2	—	—	5	—	5	—	3	6	2	11	—	1	2	1	4	—	4	8	3	15	—	4	13	3	20
20–29	4	4	25	15	48	—	—	5	5	10	4	4	30	20	58	3	5	56	14	78	2	2	8	4	16	5	7	64	18	94	9	11	94	38	152
30–39	4	15	81	27	127	—	10	10	1	21	4	25	91	28	148	1	8	37	25	71	—	1	17	3	21	1	9	54	28	92	5	34	145	56	240
40–49	5	19	112	40	176	—	2	11	3	16	5	21	123	43	192	5	4	33	16	58	—	1	6	3	10	5	5	39	19	68	10	26	162	62	260
50–59	12	23	80	31	146	—	1	8	4	13	12	24	88	35	159	1	5	14	10	30	—	1	4	2	7	1	6	18	12	37	13	30	106	47	196
60–69	6	13	60	20	99	1	3	3	3	10	7	16	63	23	109	—	1	4	4	9	—	1	6	1	8	—	2	10	5	17	7	18	73	28	126
70–79	3	9	29	13	54	—	3	1	2	6	3	12	30	15	60	—	—	1	2	3	1	—	1	—	2	1	—	2	2	5	4	12	32	17	65
80–89	—	2	11	—	13	—	1	1	—	2	—	3	12	—	15	—	—	—	1	1	—	—	—	2	2	—	—	—	3	3	—	3	12	3	18
90–99	—	—	—	1	1	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
Total	34	85	401	147	667	1	20	41	18	80	35	105	442	165	747	10	26	151	74	261	3	7	44	16	70	13	33	195	89	331	48	138	637	254	1078
I and II	66	137	642	256	1101	2	36	82	33	153	68	173	724	289	1254	15	66	311	193	585	7	25	107	63	202	22	91	418	255	787	90	264	1142	544	2041

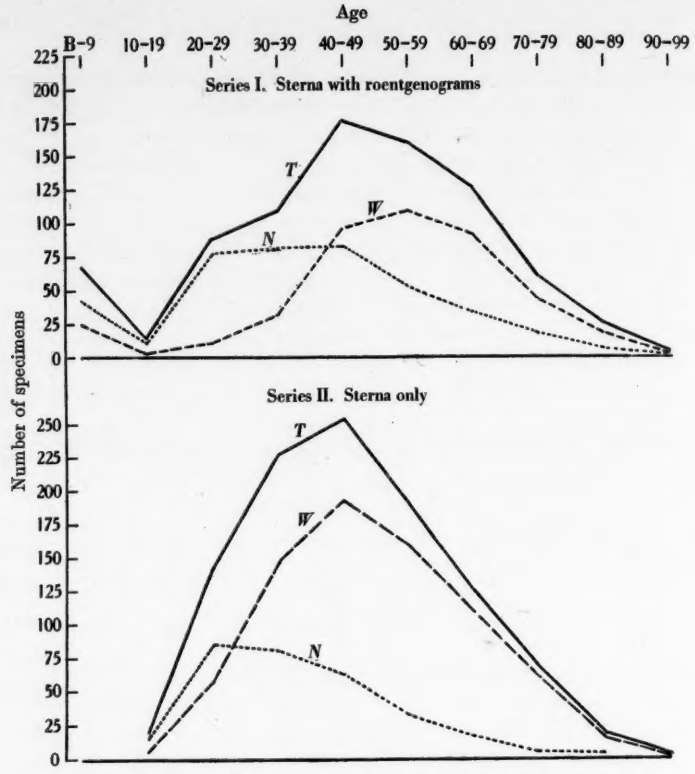


Fig. 5. Distribution of sterna according to age by decade and race.

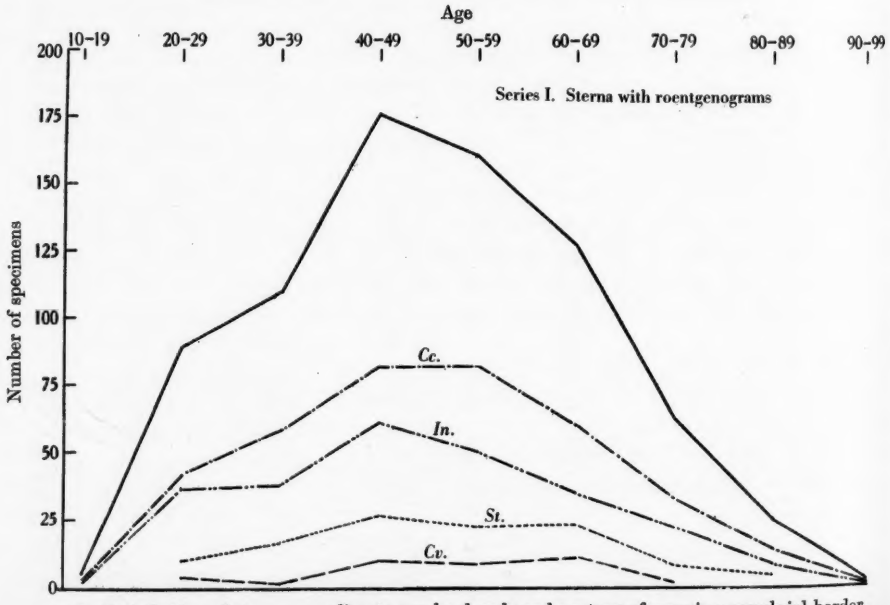


Fig. 6. Distribution of sterna according to age by decade and contour of superior manubrial border.

development in the sternum or elsewhere, and Malaguzzi-Valeri held that the mere fact that two individually rare anomalies, the suprasternalia and cervical rib, had been several times reported together was itself significant, special attention was given to such association in our specimens.

Table II shows that of the 82 sterna bearing suprasternal elements 69 or 84·1 per cent. were in all other respects normal, including 6 specimens in which manubrium and body were united, this feature not being eligible as

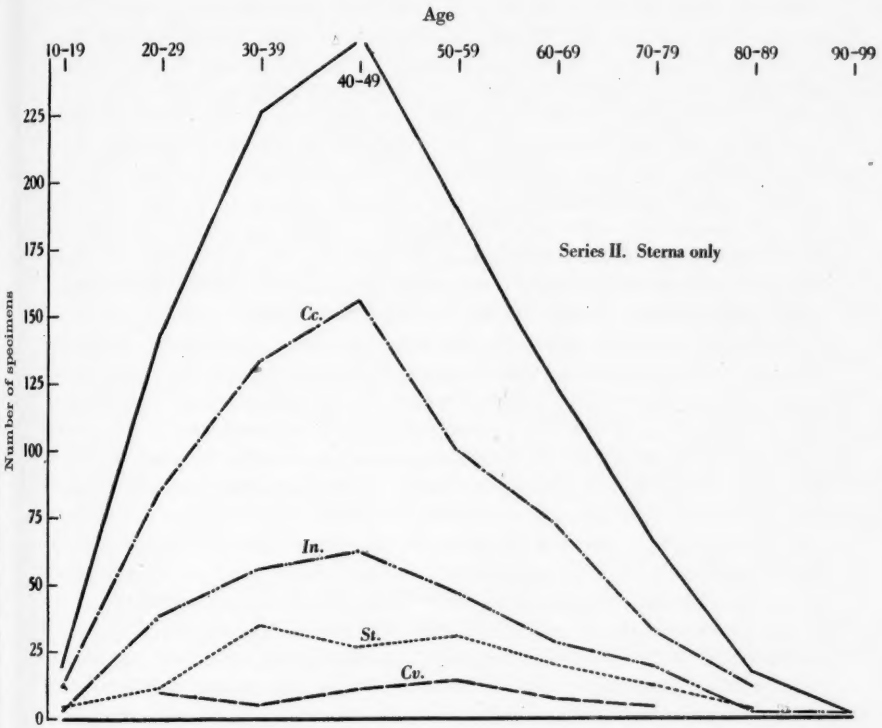


Fig. 7. Distribution of sterna according to age by decade and contour of superior manubrial border.

an anomaly. Six specimens of 7·3 per cent. had perforations of the corpus; in three, 3·6 per cent., the manubrio-corporeal joint was oblique from side to side instead of horizontal; in three sterna the body presented separate sternebrae; and one sternum, 1·2 per cent., had an unusually short and narrow corpus which articulated with only six ribs. It is quite evident then, that suprasternal bones have no particular association with anomaly or defect in the sternum.

Table X. *Incidence of manubrial types in anthropoids*

	Gorillas			Chimpanzees			Orangs		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
Concave	30	15	45	7	21	28	1	4	5
Intermediate	8	5	13	2	5	7	—	—	—
Straight	2	1	3	—	3	3	1	1	2
Convex	—	—	—	—	—	—	1	—	1
Total	40	21	61	9	29	38	3	5	8

The cervical vertebrae of 79 of the 82 cases of suprasternal bones were examined for cervical rib. In no case was this structure found, so that particular association with this anomaly must also be denied. (Of the three cases not examined, two were in maceration and one was missing.)

V. SUMMARY

The variation and incidence of suprasternal bones and the form of the superior manubrial border were investigated on 2284 human and 107 anthropoid sterna in the Hamann Museum of Western Reserve University, a larger and better documented collection of material than has hitherto been available.

Roentgenograms of 1010 of the human sterna taken before maceration and with the clavicular ligaments still attached made possible the detection of minute ossicles otherwise lost. Comparison of manubria with their roentgenograms demonstrated that the dry manubrium may show no evidence of a suprasternal bone which was present. The incidence of separate ossicles was relatively higher in our roentgenographed series than in that composed of dry bones alone. Estimates of incidence based on macerated material are, therefore, unreliable.

The facts about suprasternal bones as they now appear may be briefly stated as follows:

1. *Development.* Suprasternal bones originate in occasional lateral condensations of mesoderm from the median anlage of the sternum, which is associated with the shoulder girdle and is independent of the sternal bands and ribs. The anlagen become cartilaginous. The cartilaginous stage has been noted at six foetal months and may persist into adult life. Ossification has been observed as early as 12½ years. It probably generally occurs between the ages of 17 and 23. Ossification of the cartilage may be partial or complete.

2. *Homology.* Suprasternal bones appear to be rudiments of the epicoracoids of the primitive shoulder girdle, as evidenced by their constancy of attachment to the interarticular disc of the sternoclavicular joint, the constancy of their position on the sternum, and their homologous relationships with intermediate structures in the lower Vertebrates.

3. *Size.* Suprasternal bones range in size between that of a small shot and an average female lunate bone. An average is: length 10·4 mm., breadth 10·4 mm., height 7·3 mm.

4. *Form.* The shapes of suprasternal bones are variants of a quadrilateral pyramid having a base, four surfaces and an apex. The base articulates with the manubrium; the interarticular disc is attached to the lateral surface; the medial surface or border is joined by the inter-suprasternal ligament to its fellow or the sternum; the anterior and posterior surfaces are bound to the manubrium by the anterior and posterior suprasternal ligaments, respectively, specialized portions of the capsular ligament of the sternoclavicular joint. The apex is medially situated. Reduction in size and rounding of the margins tend to mask the characteristic form of the ossicles, but this may be discerned even in fused specimens. Most commonly the medial surface is contracted into a broad border producing a triangular pyramid.

5. *Structure.* Suprasternal bones are similar in structure to the sternum. They consist of cancellous tissue surrounded by a thin layer of compactum.

6. *Position.* Ossicles of all types occupy a constant position on the posterior manubrial border, adjoining and many times confluent with the clavicular articular surfaces but sometimes separated from the latter by a groove, which serves for ligamentous attachment.

7. *Variation.* Suprasternal bones occur in pairs and singly. They may be separate, forming a diarthrodial joint with the manubrium complete with articular cartilage and synovial membrane; they may be united to the manubrium by synchondrosis; or as small nodules they may be ensconced within the suprasternal ligaments and have no contact with the manubrium. The ossicles exhibit various degrees of fusion with the manubrium and with each other, almost complete incorporation in the former being sometimes detectable. When fusion occurs can only be surmised but is probably early. Paired suprasternal bones may be of subequal size and symmetrical form and position on the manubrium, or of unequal size and asymmetrical arrangement. One element may be separate and the other fused.

8. *Incidence.* The roentgenograms showed separate or fused suprasternal bones in 6·8 per cent. of 544 adult whites and 2·2 per cent. of 466 adult Negroes. The incidence was highest in 79 white females, 7·6 per cent.; lowest in 132 Negro females, 0·0 per cent. (One case of ossicles in a female Negro was found in the second series.) For the males the percentages were: 465 whites, 6·7; 344 Negroes, 3·0. Thus the ossicles appear three times more frequently in whites than in Negroes. They are more than twice as frequent in white males as in Negro males, and nearly eight times more frequent in white females than in Negro females.

9. *Frequency of types of ossicles.* About two-thirds of the suprasternal ossicles which occur are separate and one-third fused. Paired ossicles are slightly more frequent than single in the separate group, but paired and single are of approximately equal frequency among fused ossicles. The separate ossicles occur about 30 per cent. more often in whites than in Negroes.

10. *Classification of manubrial types.* The superior manubrial border may be convex, straight or concave. A convex border may be produced by the

fusion of suprasternal bones, or it may support separate ossicles. A concave border may be encroached upon by bony shoulders which serve for the attachment of the meniscus and at the edges the sternoclavicular ligaments. Sterna with such shoulders are classed as intermediate.

11. *Significance of manubrial types.* The human sternum with convex manubrial border and well-developed separate ossicles retains the greatest number and quantity of primitive shoulder-girdle elements and must be considered the primitive type, while the markedly concave sternum represents the greatest reduction in primitive elements and is hence the most advanced. Interpolated in descending order from advanced to primitive are intermediate, straight and convex types, it being recognized that the intermediate may be interpreted both as a regressive straight type or a concave in which the jugular notch is secondarily rebuilt.

12. *Frequency of manubrial types.* Convex and straight manubrial borders occur in greater proportion in whites than in Negroes, while the concave form is 7·5 per cent. more frequent in Negroes, both concave and intermediate types being grouped together as concave. This fact, coupled with the greater incidence of ossicles of all types and the greater proportion of separate ossicles in the whites, seems to indicate that the Negro has progressed further than the white in simplification of the upper end of the presternum.

13. *Age incidence of ossa suprasternalia and manubrial types.* The 82 ossicles and tubercles encountered in this study all occurred in adults. The age curve of these individuals compared with that of the series as a whole revealed nothing significant. The age curves of the several types of manubrial contour revealed no particular age incidence for any type such as Graves found for the vertebral border of the scapula.

14. *Associated anomaly.* The suprasternal elements in this series revealed no particular association with any anomaly or defect in the sternum, and were in no case associated with a cervical rib.

15. *Width of superior manubrial border.* The distance along the superior manubrial border between the clavicular articular surfaces has a greater tendency to be wide relative to the breadth of the manubrium in males than in females.

16. *Anthropoids.* In the anthropoid series of 61 gorillas, 38 chimpanzees and 8 orangs no suprasternal ossicles or tubercles were found, but their superior manubrial border was classified in the same way as the human. No gorillas and chimpanzees and but one orang had convex manubrial borders, but 75 per cent. of these animals had concave borders. This suggests further progress than Man has made away from the primitive type of sternum.

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CROSSED ECTOPIA OF THE KIDNEY AND ITS POSSIBLE CAUSE

By ALICE CARLETON

Department of Human Anatomy, University of Oxford

Crossed ectopia of the kidneys is a condition in which, while the ureters end normally one on either side of the bladder, one kidney has migrated to the opposite side where it lies close to, or more often wholly or partially united to, its fellow. This abnormality has also been described under the heading of "unilateral kidney". But this term is ambiguous since fusion is not always present, for Pagel (5) found separation in 9 out of 59 cases. And the term "unilateral" applies equally to a solitary kidney with suppression of the other organ.

In the case here recorded the individual was an aged male dissecting room subject with no history of renal trouble. On the left side of the abdomen a mass of renal tissue was found extending from the top of the 1st to the bottom of the 4th lumbar vertebra. It appeared at first to be a single organ, but dissection revealed a small, hypoplastic, left kidney (see drawing) with a firm, fibrous union between its lower pole and the outer and upper end of a larger right kidney. When hypoplasia occurs it more commonly affects the crossed than the uncrossed organ (Eisendrath & Rolnick (2)).

The length of the whole mass was 14 cm., of the left or uncrossed kidney 6·8 cm., and of the right or crossed 10 cm. The hilum of the left kidney was ventromedial, while that of the right kidney was directed ventrolaterally. From the upper or left kidney, three major calyces unite to form a very large pelvis narrowing abruptly to the ureter. The pelvis of the lower kidney is normal in size. The suprarenal glands are normal in form and position.

Congenital idiopathic dilatation of the ureters was present (see Fig. 1), to a slight extent on the right side, markedly on the left. In the latter the ureter is at first very narrow, then wide. Lower down, as it crosses the external iliac vessels, and again previous to its entry into the bladder, it shows two segmental dilatations. According to Beer & Hyman (1) this condition has been variously ascribed to a developmental defect of the muscular wall, a disturbed nervous mechanism, and a dystrophic condition of the urinary tract. It has been compared to Hirschsprung's disease of the colon. The dilatation in this case affects the wall unequally, more on the lateral than on the medial side, and suggests a muscular deficiency. At its lower end the right ureter makes a marked curve and opens into the bladder wall more posteriorly and more centrally than its fellow on the left.

Multiple small diverticula of the bladder were found. This is generally considered an acquired and not a congenital condition—a herniation of the epithelium through a weak muscular wall. The segmental dilatation of the left ureter suggests an inborn muscular defect in both parts.

Fig. 1. The kidneys have been vertically rotated so that their ventrolateral surfaces are directed ventrally in the drawing. The space between the two kidneys on their medial side was filled with vessels.

ARTERIAL SUPPLY

One artery supplies the upper kidney. It arises from the lateral aspect of the aorta just below the level of origin of the superior mesenteric. Three arteries supply the lower ectopic kidney. Two come off the aorta side by side, half an inch above the bifurcation, just below the level of the inferior mesenteric. The right one goes to the front, the left to the back of the kidney, whose

left margin it incises deeply. A small branch of the left common iliac artery passes behind the kidney, causes a second indentation in its left border and curls round to the anterior surface to end behind the renal pelvis.

The aorta bifurcates on the 3rd lumbar vertebra. The left common iliac artery runs a highly tortuous course, overlapped in part by the renal mass.

VENOUS DRAINAGE

A normally placed left renal vein drains the fixed left kidney, and receives the left suprarenal and spermatic veins. It communicates freely with a lumbar azygos vein just before this pierces the left crus of the diaphragm. Several large venous tributaries on the front of the ectopic kidney unite to form a vein which crosses the bifurcation of the aorta to end in the inferior vena cava. From the upper indentation on the left border a small vein passes behind the kidney to the lumbar azygos vein.

The inferior vena cava is separated from the aorta by a space of over half an inch. It is formed on the 4th lumbar vertebra. The left common iliac vein is much larger than the right.

It may be mentioned that there is a psoas parvus on each side. On the left, its tendon sends a large aponeurotic expansion across the iliac fossa to be attached to the iliac crest (see Fig. 2). This expansion has a free crescentic upper border. The descending colon lay behind the kidney and sent a U-shaped loop into the pocket so formed. A similar condition was shown to the Anatomical Society by Jacobs in February 1934.

CASES HITHERTO RECORDED

Crossed ectopia is one of the rarer renal abnormalities, and is not often mentioned in anatomical text-books. Beer & Hyman (1) estimate it as occurring in 1 in 8000 cases. Stewart & Lodge (7) found 1 in 6500 autopsies. In 1934 Eisendrath & Rolnick (2) found 70 verified cases in all, up to that date. It occurs with about equal frequency on the right and left sides. As in other renal abnormalities, there is a preponderance of males (39 out of 55 cases, Pagel (5)). Concomitant genital abnormalities were found in 12 out of 55 cases, e.g. suppression of one-half of the uterus and its tube, failure in development of the ductus deferens, seminal vesicle, and ejaculatory duct of one side. Such abnormalities are much more commonly associated with congenital solitary kidney.

Some fusion is nearly always found in crossed ectopia. It may be a firm fibrous union or an actual mingling of renal tissue. The relation of the two organs is that, as a rule (52 out of 59 cases), the uncrossed kidney lies cranial to the crossed one, placed either end to end or overlapping. The two may form a long oval, an S-shaped or an angulated mass. Some malformation and lobulation is the rule. The hilum of the uncrossed kidney is most often medial, that of the crossed kidney ventral or ventrolateral, but much variation is found. The ureter from the crossed kidney passes to the opposite side. The ureter from the

uncrossed organ if, as usual, it is the upper one, may pass in front of, or medial or lateral to the mass.

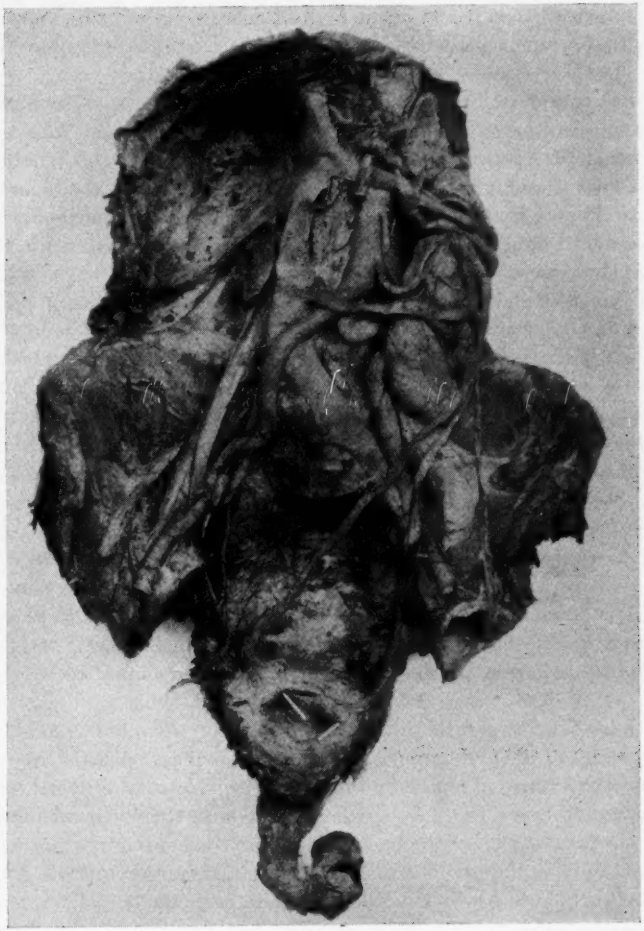

Fig. 2. The kidney mass *in situ*. The left suprarenal is seen above and medial to the left kidney. The left iliac fossa is partly hidden by an expansion of the psoas parvus tendon.

ORIGIN OF ABNORMALITIES INVOLVING RENAL FUSION

Very little information is to be found in the literature on this subject, beyond the obvious fact that the condition is due to a fusion of the developing renal masses. Linberg (4) states that there are two types of fused kidney, according to the stage of development at which the abnormality occurs. If it

happens before the kidney has left the pelvis, the united mass is held down "an Ort und Stelle" by the inferior mesenteric artery. If the union occurs at a later stage, the kidneys reach a normal level, but have a horseshoe form. We may state here that it seems in the highest degree unlikely on embryological grounds that a union of the renal masses ever occurs after they have left the pelvis.

A brief consideration of the embryology, based largely on Schreiner's studies, suggests various possibilities. In the 5-mm. human embryo of the fourth week, the ureteric bud appears at the angle where the Wolffian duct changes from a cranio-caudal to a ventral course to enter the cloaca. It is important to note that in its earliest state the bud is a dorsomedial projection from the duct, though it very soon shifts laterally. Furthermore, it grows at first directly backwards towards the vertebral column. In doing so it develops a blind, expanded end on a narrow stalk, and this blind end, in turn, pushes out cranial and caudal diverticula.

Medial, both to the Wolffian duct and to the ureteric bud when it first appears, lies the undifferentiated cell mass of the Wolffian body. When the mesonephros forms within this, the undifferentiated tail of the mass extending over three segments (29th to 31st in the rabbit) remains to form the future metanephros. By the time the ureteric bud has developed an expanded end, changes have occurred in this mass. Degeneration of its cranial end has caused its severance from the mesonephros. Around the ureteric expansion, an inner zone of closely packed cells with numerous mitoses has formed. This in turn is surrounded by a mass of more loosely packed cells—Schreiner's (6) "outer zone". The outer zone is at first extensive, touching the mesonephros at its upper end, but it shrinks gradually to become a thin covering for the inner zone, and in the course of time forms the capsule and connective tissue of the kidney.

In the earliest stage, therefore, the backwardly directed ureteric diverticula lie lateral to the undifferentiated metanephric mass. A little later the cells surround the expanded end. The buds and their cloud of cells are separated from one another by the very small hindgut near their ventral extremities, but for the greater part of the backward course the loose tissue behind the gut is the only intervening structure. By the 8-mm. stage, the ureteric diverticula and their surrounding cells have shifted laterally to the dorsolateral aspect of the Wolffian duct.

It would, therefore, appear probable that renal abnormalities involving fusion must occur between the 5- and 8-mm. stages. Union limited to the outer zone of cells would produce a fibrous connexion; involvement of the inner zone of cells a mingling of actual renal tissue. Since the lower ends of the cell mass are nearer together than the upper, the commonest form of fusion, as we should expect, is a union of the kidneys by their lower poles. Now a consideration of the reports of the different types of fused kidneys, horseshoe-shaped, sigmoid, L-shaped, "cake" or crossed ectopic kidney, strongly suggests that the direct

ascent of a large kidney mass in the midline is soon blocked, presumably by the vessels passing to the gut. Union of the lower poles allows the developing kidney masses to grow up on either side of the midline. But the bar of union hinders ascent to the normal level. It is rare to find this bar above the 4th lumbar vertebra. The greater the degree of union, the greater the difficulty of ascent, so that a "cake" kidney, where the medial borders have fused, is rarely found higher than the sacral promontory.

In examining a series of rabbit embryos, Schreiner (6) noted in many cases an inequality of growth rate between the ureteric diverticula of the two sides, with a tendency of the left to lead. Schreiner (6) found such close resemblance between human and rabbit embryos at this stage and in this region that a similar inequality presumably occurs in man. It is therefore suggested that a crossed ectopic kidney arises when fusion occurs between developing masses which show an unusually high degree of difference in growth rate. Should the cells round the lower diverticulum of one ureteric bud unite with those round the upper diverticulum of the other, an end to end or overlapping fusion will result. As we have seen, direct median ascent is barred. The mass must therefore grow up on one side or the other. In the majority of cases the upper kidney leads the way to its own side. When the reverse happens it might be due to an asymmetrical union, so that ascent on the side of the lower kidney offered less obstruction. All grades of ascent have been found, and the lower kidney may never cross the midline completely, but remain centrally placed on or below the pelvic brim. This has been called a sigmoid variety of horseshoe kidney, but is more truly a crossed ectopic kidney which has failed to complete its crossing.

The factor which causes renal union is not obvious. One suggestion, however, may be made. The umbilical artery arises at first by multiple roots which wander caudally and at the same time diminish in number. In the 5-mm. human embryo, according to Keibel and Mall (3), the artery arises by a ventral and two lateral roots upper and lower, between which lies the Wolffian body as in a ring. In the 7-mm. embryo the ventral root was found degenerated, and one root remained lateral to the Wolffian duct as the main stem of the umbilical. It would appear possible that the unusual persistence of the arterial ring or of a low lateral root to the umbilical artery, might "hold up" the lateral swing of the ureteric bud for a sufficient length of time to cause union of the developing cell masses.

SUMMARY

1. A case of crossed ectopia is described, and a brief summary given of the findings in previous cases.

2. A theory is put forward to account for anomalies of renal fusion. They occur, it is suggested, between the 5- and 8-mm. stages. If the metanephric masses are then at the same level, the result is a horseshoe or cake kidney,

depending on the extent of fusion. If one metanephric mass is more developmentally advanced than the other, the fusion results in crossed ectopia. Abnormalities of origin of the umbilical artery might provide the primary cause.

I wish to thank Miss Elizabeth Frazer for the drawing, and Mr W. Chesterman of this department for the photograph.

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SOME ABNORMALITIES OF THE ADRENAL GLAND OF THE MOUSE WITH A DISCUSSION ON CORTICAL HOMOLOGY

By H. WARING, M.Sc. and E. SCOTT, B.Sc.
University of Liverpool

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I. INTRODUCTION

In the course of a seasonal survey of the adrenal gland of the mouse, now in hand, fourteen cortical nodules have been observed and one double gland. The fourteen nodules were borne by eight glands.

In our material, as in Whitehead's (1932), the majority of the nodules are found at the venous pole. Six nodules are situated actually at the venous pole (glands 7, 23X, 38X and 48X) and three are slightly to one side (glands 41X and 38); two are situated to one side of the paravenous pole (gland 31X) and three (gland 23) are in an intermediate position.

Whitehead (1932) examined 477 glands and found fifteen to be nodulated. Of these latter eight were from males, seven from females. We examined 100 glands (fifty from males and fifty from females[1]) and found eight to be nodulated, six from males and two from females.

II. TECHNIQUE

The glands were excised from chloroformed animals, fixed in Bouin, paraffin embedded and sectioned at 5 μ, then stained in Ehrlich's haematoxylin and eosin. All glands were serially sectioned, and the size estimation given in the case of the double gland has been arrived at by the method of Jackson (1919) and Deanesly (1931).

[1] It is not quite clear how many of the 477 glands examined by Whitehead were from males and how many from females. He states that the glands came from 243 mice, and that 112 were males and 121 females. It looks as though there is a mistake in the printed figures.

III. THE NORMAL ADRENAL GLAND OF THE MOUSE

Sections of a gland from a mouse 4 weeks old, of either sex, show an outer zona glomerulosa, a zona fasciculata and a large zone of highly eosinophil cells interlocking with the medulla. At 30 days, in the male, the interlocking zone rapidly degenerates, leaving a connective tissue sheath between the medulla and the permanent cortex. At the same time the inner ends of the fasciculate columns assume the form of a zona reticularis. In the female, also, the interlocking zone degenerates and a zona reticularis is formed, but in this sex the process is more protracted, and while it may commence at 30 days, it usually terminates at a very much later period (Miller, 1927). The histological details have been described by Masui and Tamura (1926), Miller (1927), Deanesly (1928), Whitehead (1933, *a*, *b*) and Waring (1935).

Masui and Tamura considered that the interlocking zone in the mouse adrenal was a true reticulate zone. They described it as appearing post-natally between the medulla and the fasciculate columns. Miller gives a similar account of its first appearance, but, recognizing the irregularity at the base of the fasciculate columns as a true zona reticularis, she called the interlocking zone "*X*", pending further investigation. Deanesly followed Miller in calling the interlocking zone "*X*", but considered that the true zona reticularis of the late adult glands was the remains of the interlocking zone—the zona reticularis and the interlocking zone would thus be one and the same tissue. Waring (1935) showed that ontogenetically the interlocking zone is the remains of the original cortical anlage and considered that, as such, it is the homologue of the boundary zone in Man and in the cat.

Thus in young mice of both sexes, up to 30 days, sections of the adrenal gland show an outer zona glomerulosa, a zona fasciculata with a slight irregularity at the base of the columns in later stages, and a large eosinophil zone interlocking with the medulla. After this time the story is different in the two sexes, but eventually, in both, the interlocking zone degenerates, leaving only a connective tissue band between the cortex and the medulla, and the irregularity at the base of the fasciculate columns becomes a zona reticularis.

IV. ACCESSORY ADRENAL BODIES

A. Literature

(*a*) *General*

Accessory adrenal bodies have been reported from various Mammals, including Man. Sometimes they are entirely cortical, sometimes entirely medullary (chromaphil), or they may contain both cortical and medullary elements. The present paper concerns only purely cortical bodies and one accessory body containing both cortex and medulla.

According to Swale Vincent (1924) and Sharpey-Schafer (1924), accessory cortical bodies are to be found near the chief adrenal, in various parts of the

abdomen, and particularly in the genital region. Soulié (1903) mentions that the accessory body may be embedded in the cortical substance of the main gland. Apparently the accessory bodies may retain an embryonic cortical condition (Soulié) or may show a differentiation into three zones (Soulié and Swale Vincent). The origin of these bodies is unknown, but the postulate of Soulié appears sound. He says "certains amas épitheliaux peuvent, pendant les premiers stades du développement se trouver isolés par une enveloppe mésenchymateuse et conserver des rapports plus intimes avec les organs voisins de leur lieu de formation qu'avec la capsule principale elle-même". It will be shown later, however, that some cortical nodules, both from their position and from their constitution, appear to have originated from the main gland later in its development—a view which is in harmony with one suggestion put forward by Zuckerkandl (1912). Zuckerkandl considers that the theory of the formation of accessory bodies from special buds (as suggested by Soulié) lacks evidence and thinks that, in general, accessory masses represent separated portions of the parent organ. In support of this latter view Zuckerkandl quotes Wiesel (1902, 1907) as having shown that sympatho-chromaffin immigration in Man produces a cleavage of the gland as a result of which portions are separated off. The subsequent migration to diverse parts of the abdomen would then take place in association with other organs.

In Aichel's opinion (1900) those accessory bodies associated with the genital glands arise from portions of retrogressing mesonephros.

There is little information regarding the form and distribution of true accessory adrenals containing both medulla and cortex. Swale Vincent describes their occurrence in unspecified Mammals "in the neighbourhood of the abdominal sympathetic and in the region of the body where the cortical elements first arise". It would appear that they are inconstant in appearance, for Zuckerkandl reports an examination of fifty children in which only one showed an accessory body with both medullary and cortical tissue. Neither Soulié nor Sharpey-Schafer describe clearly the topographical position of such accessory bodies. Zuckerkandl describes how in true accessory glands the medulla may be central, or on one side of the cortical elements, or how the two may interpenetrate. He does not mention the constitution of the cortex where it is peripheral to the medulla and where, therefore, one would expect the accessory body to be a diminutive copy of the principal organ. He considers that these accessory glands arise by a splitting off, from the main gland, of cortex and medulla, and that when the medulla is central it is due to subsequent migration of the medullary substance into the centre.

A slight difference of opinion in regard to these true accessory adrenals is expressed by Soulié (1903), who considers that "Cette variété peut provenir d'une partie détachée de l'organe principal à un stade assez avancé du développement, ou plus probablement de la pénétration dans une capsule accessoire corticale de quelques cellules parasympathiques."

(*b*) *Mouse*

In the literature dealing with the adrenal gland of the mouse we have been unable to find any record of a true accessory adrenal gland containing both medulla and cortex. There are, however, three records of accessory cortical tissue: Slye, Holmes and Wells (1921), Löwenthal (1931) and Whitehead (1932).

Slye, Holmes and Wells (1921) in a description of tumours in the adrenal gland of mice describe a single case of "cortical adenoma of a misplaced interrenal rest—the tumour nodule being composed of a solid mass of cells resembling those of the adrenal cortex except in lack of orderly arrangement, closely packed together and flattening out a thin shell of adrenal cortex, evidently all that remains of an adrenal rest". They state that in all respects this tumour corresponds to the simple adenoma of the adrenal cortex so commonly found in Man.

Löwenthal (1931) described small lipoid-filled "Rindenadenoma oder akzessorische Rindennötchen", the structure of which does not materially differ from that of the cortex of the main gland.

Whitehead (1932) has described and figured nodules of accessory cortical tissue in the adrenal gland of the mouse. He described them "as round masses of cells like those composing the bulk of the cortex and containing sudanophil lipoid". He gives no further histological details. All, except two, of the cortical nodules discussed in the present paper seem to be of the type described by Whitehead in so far as they consist entirely of tissue like that of the permanent cortex. The two exceptions (glands 48X and 23X) are nodules containing both permanent cortex and a tissue which *looks like interlocking zone*, but which may be degenerating permanent cortex.

We are unable to make any statement in regard to the cell contents of these nodules because the abnormalities were only discovered after the tissue had been prepared for other purposes by an entirely different technique from that used by Whitehead.

B. Description of cortical nodules and double adrenal in the mouse

The nodules herein described form a series ranging from small structures composed of relatively few cells all similar to each other in structure and size, to large nodules which show a high degree of histological differentiation. The nodules vary in regard to the intimacy of their connexions with the main gland—in some they are so far embedded in the major cortex that they do not interrupt its smooth contour: in the majority of cases they are only partly sunk in the substance of the main gland; and in two cases the nodules lie outside the main gland, one with its surrounding connective tissue just touching the capsule of the main gland, the other completely separated from it by fatty connective tissue. In the following description all the nodules associated with one gland are treated together. The glands are arranged in order of the increasing complexity of their most complex nodules.

Gland 7 has a small nodule, of approximate diameter 0·09 mm.[1] It is completely ensheathed in its own connective tissue and lies almost completely external to the capsule of the main gland, a few strands only of the main capsule passing over it. The nodule slightly indents the surface of the main gland and projects a little beyond its contour. Histologically it is composed of cells with little cytoplasm, these cells only differing from those of the zona glomerulosa of the main gland in their larger size.

Gland 31X possesses two small nodules lying in contact with each other and indenting the peripheral tissues of the gland. Each nodule is completely surrounded by its own connective tissue sheath, and together they lie embedded in the thickness of the sheath of the main gland.

The smaller nodule, of approximate diameter 0·05 mm., is composed entirely of typical zona glomerulosa cells, whilst the larger, of approximate diameter 0·18 mm., has a wide peripheral zona glomerulosa surrounding a mass of irregularly arranged, more granular cells whose vacuolization and eosinophil character is typical of fasciculate tissue.

Gland 38X possesses a medium-sized nodule, of approximate diameter 0·20 mm., which is partially sunk in the cortex of the main gland. It is completely ensheathed by the connective tissue capsule of the main gland, the inner layers of which pass under it, the outer layers over it.

Histologically the nodule is composed of irregularly arranged cells which are all very much alike, although at the periphery the cells are slightly suggestive of the glomerulosa type, and in the centre they have a slightly more fasciculate nature. The cells of the nodule share with those of the main gland the characteristic of more densely packed cytoplasm than is usual. The chief effect of this is to render less distinct the differentiation of the tissue into zona glomerulosa and zona fasciculata.

Gland 38 has a medium-sized nodule, of approximate diameter 0·13 mm., situated entirely outside the fibrous capsule of the gland but embedded in the fatty connective tissue which surrounds it. The nodule is completely ensheathed in its own capsule and consists of a peripheral zona glomerulosa surrounding irregularly arranged cells of a fasciculate nature.

Gland 41X has two nodules adjacent to each other. The smaller one, of approximate diameter 0·18 mm., forms only a shallow depression in the cortex of the main gland, whilst the larger one, of approximate diameter 0·19 mm., is completely separated from the gland. Both are ensheathed in their own connective tissue, but while the smaller is embedded in the capsule of the main gland, the larger, lies in the fatty connective tissue which lies external to the capsule of the gland. Both nodules show a peripheral zona glomerulosa surrounding a central mass of irregularly arranged fasciculate cells.

Gland 23 has three nodules. Two of these are in close association with each other; the smaller, of approximate diameter 0·09 mm., making no depression in the cortex of the main gland, whilst the larger, of approximate diameter

[1] Measurement of the largest microscopic section.

0·36 mm., projects slightly into the substance of the main gland. Each is ensheathed in its own connective tissue and both are embedded in the thickness of the capsule of the main gland. The smaller nodule is composed exclusively of zona glomerulosa cells, but in the larger there is a peripheral zona glomerulosa surrounding cells which only differ from typical fasciculate cells in their smaller size, and at one pole of the nodule these are arranged in well-defined radial columns.

The third nodule, of approximate diameter 0·36 mm., is elongated and flattened against the cortex of the main gland indenting its contour. It is completely surrounded by the capsule of the main gland, the fibres of which pass above and below it.

Histologically there is a peripheral zona glomerulosa surrounding cells of a fasciculate nature, which in a few places show signs of a columnar arrangement. At the two extremities of the long axis of the nodule are masses of glomerular cells partially constricted off from the rest of the nodule by ingrowths of the capsule. This arrangement strongly suggests that these masses may be incompleted stages in the formation of nodules.

Gland 23X has a large nodule, of approximate diameter 0·26 mm., completely enclosed within a connective tissue capsule which just touches the capsule of the gland (see Plate I, fig. 1). This nodule is more highly organized than any previously described. It has a peripheral zona glomerulosa internal to which are fasciculate cells arranged in well-marked columns radiating from approximately the centre of the nodule. In the very centre of the nodule and interspersed amongst the inner ends of the fasciculate columns are small deeply staining cells, with indistinct cell walls, which strongly resemble the cells of the fully developed interlocking zone in a young gland. If these cells are indeed interlocking or boundary zone cells, it is interesting to note that this cortical nodule was obtained from a male of 44 days in which, of course, the interlocking zone tissue of the main gland had degenerated.

Gland 48X has three nodules. In their vicinity the fibrous capsule of the gland is divided into an outer layer which passes over the nodules and an inner layer which passes inwards either completely or incompletely underneath them. The largest is incompletely separated from the main gland, and the smallest is incompletely separated from the largest, whilst a medium-sized one is completely separated both from the main gland and from the other nodules.

The largest of these three nodules, of approximate diameter 0·33 mm., is probably the largest of the whole series herein described. It is partly sunk in the main gland, indenting the cortex considerably, but also projecting beyond the main contour of the gland. This nodule is confluent with the main gland by an isthmus (Plate I, fig. 2) which apparently passes to one side of the main axis. Histologically the nodule shows a peripheral zona glomerulosa which appears to be continuous through the isthmus with that of the main gland. Wherever the nodule indents the main gland the zona glomerulosa of the latter

is very narrow. Internal to the zona glomerulosa are well-defined columns of fasciculate cells. Sections taken across the greatest diameter of the nodule (Plate I, fig. 3) show these columns radiating outwards from a point situated excentrically below the middle of the nodule. Sections taken through the isthmus (Plate I, fig. 2) show what is probably a similar arrangement except that here the picture is complicated by the presence of very deeply staining cells. These cells extend from the connective tissue separating the medulla from the cortex in the major gland, through the region of the zona fasciculata into the substance of the nodule. In the plane of the isthmus they appear as a wide tract (Plate I, fig. 2) but in sections at the largest diameter they are confined to isolated groups in the centre of the nodule (Plate I, fig. 3).

Such very darkly staining cells are somewhat suggestive of the interlocking zone cells—they stain deeply, their cytoplasm is closely granular, and in this particular instance their nucleo-cytoplasmic ratio is high. In addition to this they are in direct connexion with the connective tissue band surrounding the medulla of the main gland, and this connective tissue had, prior to its degeneration, existed as interlocking tissue between the cortex and the medulla. Nevertheless, they are more deeply stained than normal interlocking cells. Also in the gland under discussion the interlocking zone has elsewhere completely degenerated. It seems more probable that they represent degenerating permanent cortex, and in some places, where they abut on to normal fasciculate-type cells, columns do exist with alternate very dark cells and normal fasciculate cells, as though the tissues were in a state of transition. In fact, in many places, there are also in the same columns deeply staining but unmistakably fasciculate-type cells which are obviously intermediate in condition between the normal fasciculate and the very deeply staining type under discussion. Exactly similar darkly staining cells of both types are not infrequently found in otherwise normal glands (Plate I, fig. 4 and Plate III, fig. 10). They occur in irregular patches anywhere throughout the width of the permanent cortex. The nucleo-cytoplasmic ratio in both these types of darkly staining cells varies considerably, so that either may have exactly the same ratio as is found in the normal fasciculate cells adjacent to them; or the nucleo-cytoplasmic ratio may be higher, in which case the intermediate type cells almost exactly resemble normal zona reticularis cells; or the ratio may be very high, in which case the most darkly staining cells superficially resemble interlocking zone cells.

The smallest nodule, of approximate diameter 0·08 mm., is composed of small cells which show very little histological differentiation. The majority are typical cells of the zona glomerulosa, but there are also a few cells of the darkly staining type described above.

The medium-sized nodule of approximate diameter 0·11 mm. is composed exclusively of large glomerular zone cells.

The double adrenal

In all the cases described above the accessory bodies were composed exclusively of cortical tissue. In gland 18 about to be described, taken from a female 30 days old, the accessory body possesses also a medulla completely separate from that of the main gland, so that the whole structure forms a double gland (see Plate II, figs. 5 and 6). This double gland is considerably larger than glands from other animals of the same age and sex. The accessory gland constitutes about one-seventh of its total volume, and this is evidently truly additional tissue for without it the main gland would still be about the same size as its sister gland from the opposite side. Even without the accessory body, however, the glands of this female are appreciably larger than those of any other female of the same age which we have examined. Apart from the adrenals she appeared to be quite normal.

The accessory gland is situated at the venous pole and is not completely separated from the main gland. The fibrous connective tissue is well defined around three-quarters of the major gland. At the junction of the two glands, however, the sheath splits, the innermost layers passing inwards at right angles to the surface for a short distance so as partially to cut off the accessory body, whilst the outermost layer continues its course over the accessory body. In this region the outermost layer has few nuclei and is thin, suggesting that it has been stretched by the growth of the accessory body within it.

The major gland

The major gland is perfectly normal except in that region where it is confluent with the minor gland (see Plate II, figs. 5 and 6).

The permanent cortex is composed of a narrow zona glomerulosa internal to which are well-defined columns of typical fasciculate cells. Internal to the zona fasciculata is the interlocking or boundary zone, whilst in the centre is the medulla. All three regions are in a normal condition for a 30-day-old female, except where the two glands are confluent. In this region, however, there is a mass of syncytial tissue entirely devoid of cell boundaries, in place of the well-defined fasciculate columns of the permanent cortex (see Plate II, fig. 6). This syncytial tissue lies completely across the connecting region of the two glands. It also passes into the accessory gland for some distance around the periphery replacing the permanent cortex in part and gradually merging into true fasciculate tissue. The cytoplasm of this syncytial tissue (see Plate III, fig. 8) is granular and slightly vacuolated with a few scattered large vacuoles. It is eosinophil and is much more deeply stained than the normal lightly staining fasciculate tissue of the permanent cortex. The irregular shape of the nuclei, their deeply stained, crushed appearance, their excessive numbers in places and the absence of cell boundaries all suggest that degeneration is taking place. And since this syncytial tissue merges into both

true cortical and boundary zone tissue, and in part replaces them, it probably represents both these tissues in a degenerating condition.

Where the syncytial tissue merges peripherally into the fasciculate tissue of the major gland the fasciculate cells show certain distinctive features. Firstly, they are more frequently in a reticulum than in columns; secondly, their cytoplasm is less vacuolate than is normally the case; thirdly, the tissue stains more deeply than usual with eosin; and finally, the cells have a higher nucleo-cytoplasmic ratio than the normal fasciculate cells. These cells are exactly similar to the modified fasciculate cells described above under gland 48X (p. 305), and, as already pointed out, they are very like ordinary reticular zone cells. In the gland now under discussion they seem to be intermediate in character and often in position also between normal fasciculate cells and syncytial tissue (cf. Plate III, fig. 8), but they do not invariably abut on to syncytial tissue (Plate III, fig. 9). For the most part the syncytial tissue appears to have the same cytoplasmic structure as this dark fasciculate tissue; but its vacuolization is less regular, and in places its cytoplasmic granules are more concentrated so that it stains darker than the dark fasciculate tissue, whilst in other places there is a higher degree of vacuolization than in the dark fasciculate tissue and the staining is correspondingly lighter (Plate II, fig. 6).

The interlocking zone of the major gland immediately internal to the syncytial tissue is considerably modified. Firstly, it is about half as broad as the rest of the boundary zone (Plate II, figs. 5 and 6). Secondly, the cell contents are even more highly eosinophil than those of the rest of the boundary zone cells. Thirdly, the cells do not form a compact tissue, rather do they form a meshwork, the interspaces of which are devoid of any tissue. The possibility suggests itself that this meshwork may be due to shrinkage during fixation, but in view of the fact that the rest of the gland is perfectly fixed, this seems improbable. Lastly, the outermost layer of this part of the boundary zone is composed of a line of degenerating nuclei connected together by strands of protoplasm. The cells immediately internal to this show a collapsed appearance which suggests that they are about to degenerate. Hence, in this region of the interlocking zone, incipient degeneration is shown, but elsewhere in the major gland the zone is perfectly normal.

The accessory gland

The accessory gland is composed of a permanent cortex, interlocking zone and a medulla (see Plate II, figs. 5 and 6). All these regions show certain abnormalities.

The permanent cortex is irregular in thickness. The zona glomerulosa is recognisable in only two small areas—its place being taken elsewhere by either syncytial tissue or by cells of the deeply staining fasciculate type, which seem to belong to displaced fasciculate columns. The fasciculate columns appear distorted, so that instead of radiating from the boundary zone to the periphery

as in normal glands, they have come to be almost tangential to the boundary zone (Plate II, fig. 7). Furthermore, the fasciculate tissue is of two types as in the major gland. Firstly, there is the normal vacuolate eosinophil tissue, staining a light red with eosin. This type occurs only locally; it is especially evident external to the boundary zone in that region directed towards the major gland (Plate II, fig. 6). Secondly, there are many cells of the more deeply staining fasciculate tissue, the characters of which have already been described in the account of the major gland. These cells are very numerous and appear to be transitional both in structure and, in many places, in position also between the normal fasciculate cells and the syncytial tissue (Plate III, fig. 8). As already stated, these cells approximate closely to those of a normal zona reticularis. The syncytial tissue, described above, replaces a considerable amount of the permanent cortex (Plate II, fig. 6), but so far as can be ascertained it does not replace any of the interlocking zone—a fact which is in contrast to the condition in the major gland where it replaces both interlocking zone and permanent cortex at the junction with the minor gland.

The interlocking zone is composed of rows of cells radiating outwards irregularly from the medulla, and forming a meshwork, the spaces of which are devoid of any tissue. Histologically, the cells are of two types as in the major gland, but in the accessory gland it is the more highly eosinophil cells which preponderate. They occur in all parts of the boundary zone, the other less deeply staining type being few and scattered.

The medullary cells of the accessory gland are of two types, firstly, vacuolated cells staining a light blue with haematoxylin, and secondly, smaller non-vacuolate cells which stain deep purple. Both of these types occur in normal glands, but the former are by far the more common. The latter cells are scattered throughout the medulla, being especially numerous at the periphery. Some extend through the cortex almost to the periphery of the gland, as described by Whitehead (1932). The significance of the two types is not understood.

V. DISCUSSION

In the course of the present study several interesting problems have arisen.

The fact that all the fourteen nodules herein described occurred in eight glands out of a total of 100 examined shows that a nodule is not a purely "accidental" occurrence. There seems to be a tendency in certain glands to form nodules, but what cause underlies this tendency is unknown.

The idea presented itself that there might be some correlation between the formation of nodules in the adrenal and some abnormality in one of the other endocrine glands. The thyroids[1] and gonads were examined from all animals with adrenal nodules, but no abnormalities of any kind were found. We have not yet been able to examine the other endocrine glands.

The time in development at which nodules are formed and the method of

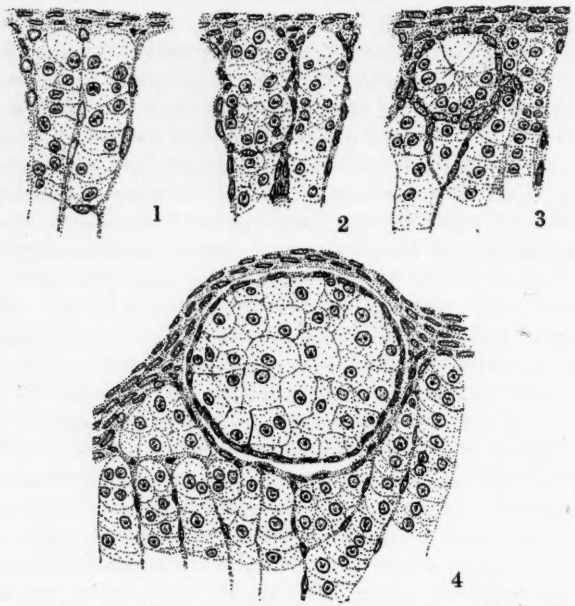
[1] We are indebted to Miss Heriot for this unpublished observation.

their formation has been much discussed. Soulié (1903) suggested that they arose by the isolation of early mesenchymal buds. Zuckerkandl (1912) thought they might be isolated comparatively late in development and accepted Wiesel's (1902) suggestion that some, at least, owe their origin to groups of cells which have become isolated by the infiltration of sympatho-chromaffin elements into the cortical anlage.

In regard to the time at which a nodule arises it seems to us that a very suggestive piece of evidence is its position in relation to the connective tissue sheath of the main gland. The cortical anlage of the mouse is constituted on the 12th and 13th day of foetal life, and on the 14th day it has a well-marked connective tissue sheath. It seems probable, therefore, that any nodule which is entirely external to the sheath of the gland has either fragmented from the main body early—before the 14th day—or has had a separate mesenchymal origin. Such nodules are found in association with glands 23X, 38X, and 41X. The remaining nodules, however, have a more intimate relationship with the main gland. In all of these except two, which are associated with gland 48X, the sheath surrounding the major gland appears to be split, so that its innermost fibres pass beneath the nodule and its outermost fibres over it. In these cases the nodules seem to have arisen from the main gland after its capsule has already formed—that is after the 13th day of foetal life. Exactly how they arise is not known. It is possible that they have been separated from the main mass of cortical tissue by the path of immigration of the sympathochromaffin cells, as Wiesel (1902) suggested for Man. In the mouse this immigration starts on the 14th day, frequently in the region of the venous pole, and the path of immigration may be strongly marked. Portions of the cortical tissue might well be isolated either by the process of immigration, or by the enlargement of the medullary vein which takes place at about this time. In either case the isolated masses would tend to lie at the venous pole internal to the capsule, exactly where the majority of the nodules described above do occur. Examination of fifteen foetal glands has failed to reveal any such isolated cortical masses, but in view of the recorded low incidence of nodules these numbers have no significance. Whitehead (1932), however, examined mice aged from 15 to 338 days, and recorded cortical nodules only from those of 85 days and over. There is some support, therefore, for the view that cortical nodules arise fairly late in development.

In the light of our own observations it seems to us probable that the majority of cortical nodules arise by an invasion of the tissues of the gland by the connective tissue of the capsule. Zuckerkandl, in 1912, suggested such an origin, but only for small glomerular nodules. Sections of a normal gland show that the inner fibres of the capsule do encroach on the matrix of the zona glomerulosa, and a portion of the cortex at the periphery of the gland might well become surrounded and isolated by such encroaching fibres and thus give rise to a small nodule. Indeed, every stage of such encroachment has been observed (see Text-fig. 1). Also in gland 48X, what appear to be two

successive stages in the isolation of nodules can be seen. The smallest nodule is barely a separate nodule at all, a few fibres only partly encompass a few cells of the main nodule and by no means isolate them. The largest nodule is almost completely cut off from the main gland by fibres running inwards from the connective tissue capsule, on all sides, but failing to meet beneath the nodule so that an isthmus remains. The double adrenal shows an almost identical condition. One stage further than this would obviously give the conditions found in the majority of the nodules.



Text-fig. 1. Series of diagrams to show varying degrees of encroachment of the connective tissue capsule on the substance of the zona glomerulosa. 1, very little encroachment of connective tissue; 2, further stages of encroachment of connective tissue; 3, showing isolation of groups of zona glomerulosa cells; 4, nodule of gland 7.

Nodules originating in this way could presumably arise at any time after the formation of the connective tissue capsule, but actually an examination of developing glands shows that there is very little invasion of the gland by the connective tissue of the capsule until the glomerular zone is truly established. It is probable, therefore, that such nodules arise from already differentiated tissue.

An extremely interesting question arises in regard to the mode of origin of the different cortical regions in the more highly differentiated nodules. If the nodules have arisen either as separate mesenchymal buds or as early isolated portions of the cortical anlage, differentiation of both glomerular and

fasciculate tissue may have taken place *in situ* from undifferentiated primary cortical cells. But if the nodules have arisen later in development, as seems probable in many cases, by the encroachment of the connective tissue capsule, they have almost certainly been isolated as masses of already differentiated cortical tissue. The question then arises as to whether the fasciculate tissue of the more differentiated nodules has been isolated as such, from the main gland, or whether it has arisen within the nodule from already differentiated, isolated glomerular zone tissue. From the evidence of the series of nodules herein described it seems probable that the fasciculate tissue within the nodules has arisen, *in situ*, from glomerular tissue, for whenever fasciculate tissue is present in a completely isolated nodule it is central and entirely surrounded by a glomerular zone. This condition could not arise by an ingrowth of connective tissue into the deeper tissues of the gland. Such an ingrowth, or any differentiation of connective tissue *in situ*, would cut off a part of the cortex with the glomerular tissue and the fasciculate tissue lying side by side. And a real constricting off of a portion of the gland, although it might bring the zona glomerulosa around a central piece of fasciculate tissue, could scarcely give the beautifully centralized condition seen, for example, in the second-described nodule of gland 23, with the fasciculate columns radially arranged. It does seem to us that in such nodules the fasciculate tissue must either have arisen *in situ*, from undifferentiated cortical tissue, or have been developed *in situ* from already differentiated glomerular tissue. From the evidence already discussed it seems probable that in many instances the latter is the case.

This point, of course, raises the much-discussed problem of the origin and nature of the different zones of the normal adrenal cortex.

The commonly observed fact of the crowded nuclei in the outer regions of the cortex and the record by some workers of degeneration in the zona reticularis have given rise to the opinion that the cells of the cortex originate near the periphery and move constantly inwards, to assume first the form of fasciculate cells, then of reticulate cells, and finally to degenerate. Gottschau (1883) was the first to formulate this conception. Hoerr (1931) has recently surveyed the literature on the subject and made new observations on the adrenal of the guinea-pig. He concludes that the cells are "continually dying off in the zona reticularis and being replaced by cells produced mainly at the border of the glomerular and fasciculate zones". Roud (1902), working on the mouse itself, was convinced of the inward migration of the cells, and in expounding this view he described gradual stages of transition in the colour of the protoplasmic granules from cyanophil in the zona glomerulosa to eosinophil in the zona fasciculata. Deanesly (1931) concluded, from her own work on the mouse, that there was little evidence of inward migration, but Whitehead (1933 *b*) has recently shown, by an analysis of the incidence of mitosis in the cortex of the mouse adrenal, that the subcapsular layer is certainly the region of most active division.

It appears to us that the present study of cortical nodules and of the one double gland may throw some light on this question.

In the first place, if nodules showing glomerular and fasciculate tissue do indeed develop, as seems probable, from isolated portions of glomerular tissue, the fact would give support to the theory of the origin of the fasciculate tissue from glomerular tissue in the normal gland. It would at least show that such an origin is possible.

Further, the stages of degeneration seen in the double adrenal gland, and probably also in the largest nodule of gland 48X, are very suggestive in regard to the normal fate of the cortical cells. If the cortical cells do arise in the zona glomerulosa, as Gottschau (1883) postulated, and move inwards, changing first to fasciculate, then to reticulate cells and finally degenerate, they must undergo a very definite series of changes—their vacuoles must be reduced and their cytoplasm concentrated, they must become small, and they must become much more deeply staining. But there is no absolute proof that these changes do take place. In the double gland, however, degeneration is undoubtedly taking place (see pp. 306 and 307). The syncytium, with its collapsed, deformed, irregularly scattered nuclei, is obviously a degenerating tissue, and where this tissue abuts on to normal fasciculate tissue the transition from one to the other is very gradual. Adjacent to the normal, large, vacuolate, lightly staining fasciculate cells are cells of a less vacuolate, more deeply staining but nevertheless fasciculate type. These darkly staining fasciculate cells pass imperceptibly into the syncytium and are almost certainly transitional stages—degenerative stages. Yet histologically they are indistinguishable from true reticular zone cells. Their position in the fasciculate zone and the manner in which they alternate in places with true fasciculate type cells indicates that they *are* fasciculate cells, but in the course of degeneration they have become to all appearances exactly like true reticular zone cells. A similar degenerative process is evidently taking place in the nodule of gland 48X, but here the end-product is an even more darkly staining completely non-vacuolate type of cell, not a syncytium. Comparable transitional stages are sometimes found in localized patches of tissue in otherwise normal glands (see Plate III, fig. 10). In these patches the normal fasciculate type cells are undoubtedly giving rise to the dark fasciculate or "reticulate" type, and these in turn to cells with even more darkly staining non-vacuolate cytoplasm, as in gland 48X, or, in at least one case, to syncytial tissue (Plate III, fig. 11).

It is fairly certain, therefore, that fasciculate zone cells, during a process of degeneration, do pass through a stage to all appearances exactly like the reticular zone cells of a normal gland. This fact, as well as the probable origin of the fasciculate type cells from those of the zona glomerulosa in some nodules, seems to us to give real support to the theory that the cells of the permanent cortex arise in the region of the glomerular zone, that they gradually migrate inwards, taking on the character of fasciculate tissue, then of reticulate tissue, and that they ultimately degenerate in the region of the reticular zone.

We wish to express our thanks to Mrs R. C. Bisbee for her constant encouragement and valuable criticism.

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EXPLANATION OF PLATES I–III

PLATE I

Fig. 1. Photograph of a transverse section of the nodule of gland 23X, showing well-defined fasciculate columns, and the centrally placed indefinite tissue which may be boundary zone. × 100.

Fig. 2. Photograph of a longitudinal section of the nodules of gland 48X to illustrate the isthmus connecting the largest nodule to the main gland. × 100.

Fig. 3. Photograph of a longitudinal section, through greatest diameter, of the largest nodule of gland 48X completely surrounded by connective tissue with well-defined fasciculate columns and the centrally placed tissue which is indefinite in nature. × 100.

Fig. 4. Photograph of a transverse section showing the contrast between the ordinary lightly staining and the darkest staining fasciculate tissues in an otherwise normal gland. ×100.

PLATE II

Fig. 5. Photograph of a longitudinal section of gland 18 through the accessory and major glands showing the two concentrations of medullary tissue. ×30.

Fig. 6. Photograph of a longitudinal section of the connecting region between the accessory and major parts of gland 18. ×60. Compare fig. 5.

Fig. 7. Photograph of a longitudinal section of gland 18 to illustrate the distorted fasciculate columns of the accessory gland and the entire absence of a zona glomerulosa. ×150.

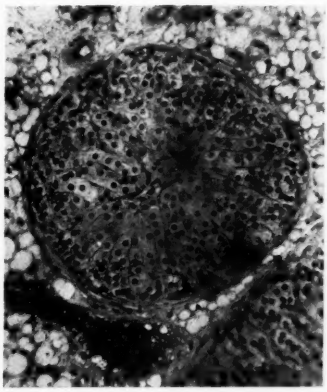
PLATE III

Fig. 8. Drawing of part of a longitudinal section of the double adrenal gland 18, to illustrate the syncytial tissue separating the fasciculate tissue of the accessory gland and the boundary zone of the major gland.

Fig. 9. Drawing of part of a longitudinal section of the double adrenal gland 18, to show normal fasciculate tissue merging into more deeply staining fasciculate tissue which is arranged in a reticulum.

Fig. 10. Drawing of part of a transverse section of an otherwise normal adrenal gland to show the three types of fasciculate tissue: (1) the normal light staining vacuolate tissue, (2) the deeply staining vacuolate tissue, (3) the darkest staining non-vacuolate tissue.

Fig. 11. Drawing of part of a transverse section of an otherwise normal adrenal gland to show light staining fasciculate tissue grading through deeply staining fasciculate tissue into syncytial tissue.



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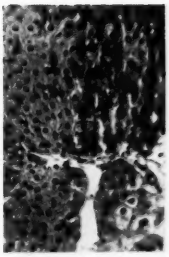

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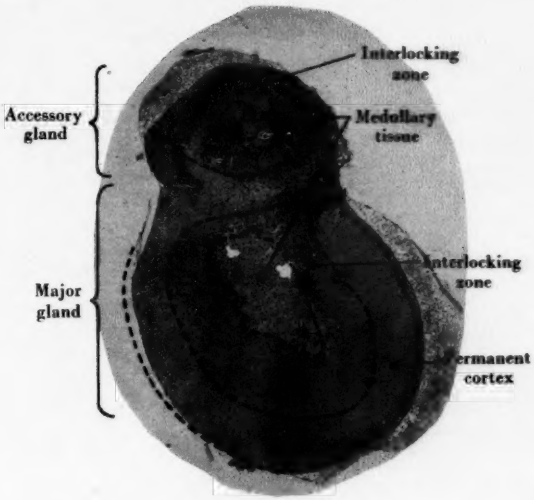

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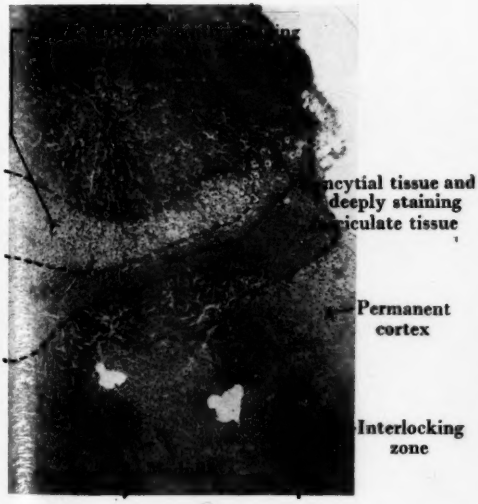

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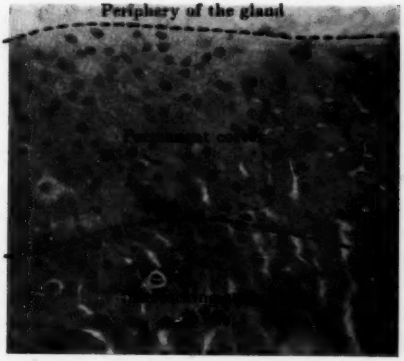

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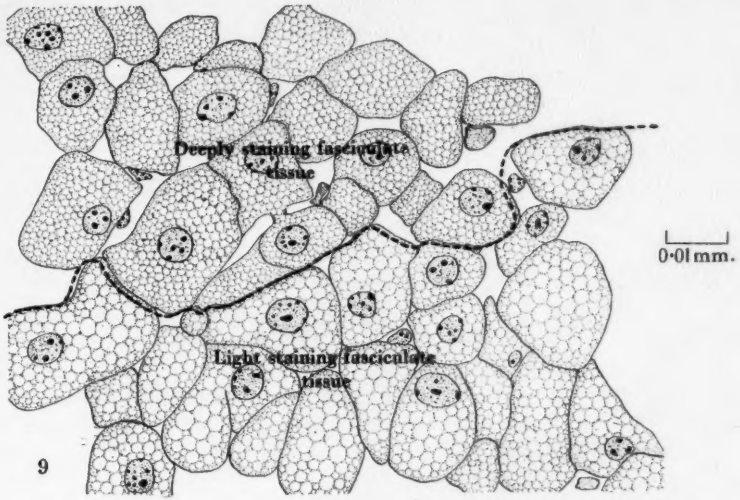

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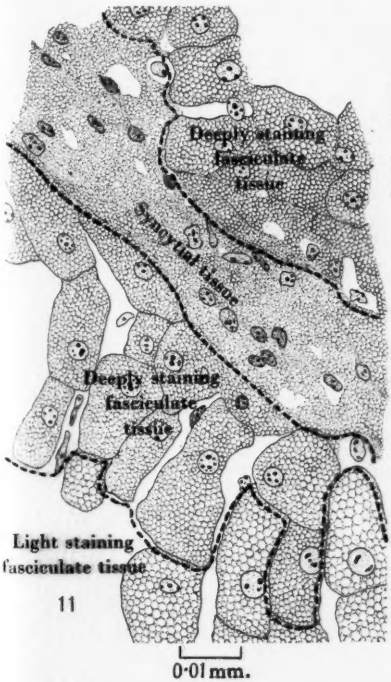

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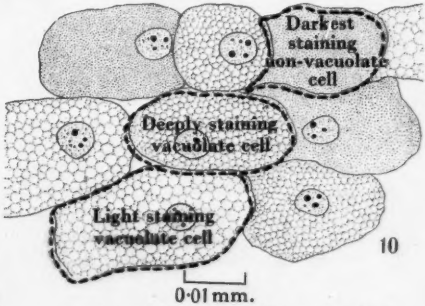

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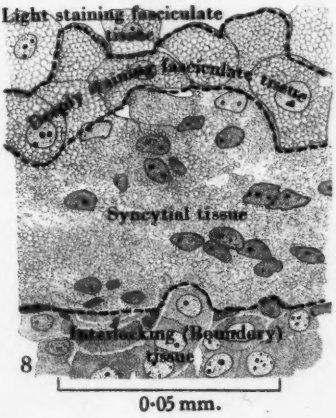

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THE DIGASTRIC MUSCLE OF *PHALANGER ORIENTALIS* AND *MACULATUS*

By F. H. EDGEWORTH, M.D.

Kohlbrugge, in 1898, stated that the posterior belly of the digastric muscle of *Perameles doreyana, Macropus brunii, Cuscus orientalis* and *C. maculatus* arises from the styloid process, but did not give any figures.

This is of some interest from a developmental point of view. The upper end of the Interhyoideus constituent of the digastric muscle in Marsupialia, which primarily has an origin from the ventrohyale, usually spreads upwards via the laterohyale to the paramastoid process. In the above-mentioned genera development stops short and the origin is from the laterohyale, which persists.

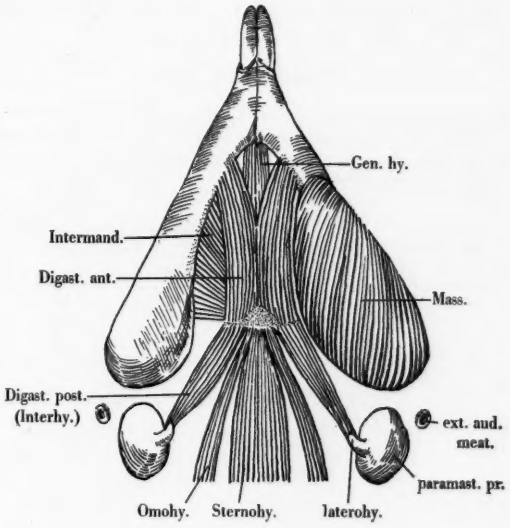

Fig. 1.

Some years ago I wished to confirm the statement in one or other of these genera but could not obtain any. Recently Lord Moyne has been good enough to bring me two specimens of *Phalanger* s. *Cuscus orientalis* from the island of Amboyna. The figure shows the condition found.

The Interhyoideus arises from the laterohyale, passes forwards, downwards and inwards, and is inserted partly by intersection into the Digastricus anterior

and partly into the transverse aponeurosis connecting the ventral ends of the Interhyoidei, which projects forwards between the oblique ends of the Digastrici anteriores.

The Digastrici anteriores, which are parallel and confluent, arise from the intersections and the aponeurosis, pass forwards and diverge outwards to the jaws.

The hindmost fibres of the Intermandibulares pass from the edges of the aponeurosis transversely outwards to the jaws. Those in front pass more and more obliquely forwards so that the foremost are almost longitudinal. They have no median raphé.

The C. hyale of the basibranchiale is the only persisting part of the ventrohyale. There is no tendon connecting it with the laterohyale. The Branchiohyoideus passes between the C. hyale and branchiale i.

In *Phalanger maculatus*, a specimen of which was kindly sent to me by Dr Burgess, of the Zoological Society, the conditions, with one exception, are similar. The cleft between the anterior ends of the Digastrici anteriores extend further backwards and the inner portions of the Intermandibulares are visible between the muscles.

ABBREVIATIONS

Digast. ant., Digastricus anterior; *Digast. post.* (*Interhy.*), Digastricus posterior s. Interhyoideus; *ext. aud. meat.*, external auditory meatus, cut across; *Gen. hy.*, Genio-hyoideus; *Intermand.*, Intermandibularis; *laterohy.*, laterohyale; *Mass.*, Masseter; *Omohy.*, Omo-hyoideus; *paramast. pr.*, paramastoid process; *Sternohy.*, Sterno-hyoideus.

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KOHLBRUGGE, J. H. F. (1898). *Arch. Anat. Physiol.*

REVIEWS

Fasciae of the Human Body and their Relation to the Organs they Develop. By EDWARD SINGER, M.D. (London: Baillière, Tindall and Cox.) 1935. Pp. xv+106, plates 24. Price 13*s.* 6*d.* net.

This work, largely in the nature of a compilation and collection, is an extensive and purely formal account of the various fasciae. It is uninformative regarding the principles of facial specialization and function, and uneven in its treatment of the several regions. Thus elaborate description is accorded the fasciae of the head and neck and of the spermatic cord, whilst the triangular ligament, the recto-vesical fascia, and the femoral sheath are very inadequately treated. There is no account of the mediastinal and peri-renal fasciae, of the meso-hepar, or Cooper's mammary ligaments, nor of the peri-vascular fascial supports of the pelvic viscera.

Illustrations of Regional Anatomy. By E. B. JAMIESON, M.D. Section VI, Upper Limb, 42 plates; Section VII, Lower Limb, 52 plates. (Edinburgh: E. and S. Livingstone). 1936. Price: Section VI, 7*s.* 6*d.* net; Section VII, 10*s.* net.

In these two additional sections the author completes his synoptic survey of regional anatomy. The illustrations provided maintain a high standard of accuracy and clarity, and are excellently produced; inevitably perhaps, this presentation in atlas form has masked their original nature and appeal as blackboard-diagrams.

A future edition would benefit by the omission of certain purely osteological plates and their replacement by illustrations of the axillary and other lymphatic glands, of the articular apparatus of the major joints, and of the bursae about the knee-joint.

The following book has been received:

A Text-book of Histology. By JOSEPH KRAFKA, Jr, Ph.D., M.D. (London: Baillière, Tindall and Cox). 1936. Pp. vii+246, Figs. 95. Price 11*s.* 6*d.* net.

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